"A STUDY OF HEART RATE VARIABILITY AND SERUM INSULIN LEVELS IN PATIENTS WITH PARKINSON'S DISEASE"

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CERTIFICATE

This is to certify that the Dissertation entitled "**A STUDY OF HEART RATE VARIABILITY AND SERUM INSULIN LEVELS IN PATIENTS WITH PARKINSON'S DISEASE"** by the candidate **Dr.S.P.Girijasivam** in partial fulfillment of the requirements for **M.D. (PHYSIOLOGY)** is a bonafide record of the research done by her during the period of study (2012 to 2015) in the Institute of Physiology and Experimental Medicine, Madras Medical College, Chennai- 600 003.

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A STUDY OF HEART RATE VARIABILITY AND SERUM INSULIN LEVELS IN PATIENTS WITH PARKINSON'S DISEASE

ABSTRACT

Degree for which submitted:		Doctor of Medicine(MD) in Physiology
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BACKGROUND: Parkinson's disease is the commonest neurodegenerative illness affecting the world population. Autonomic dysfunction and non-motor symptoms (NMS) in Parkinson's disease (PD) are frequent, disabling and reduce quality of life of patient. The prevalence of insulin resistance might play a role in the etiopathogenesis of Parkinsonism.

AIM AND OBJECTIVE: There is a paucity of studies on autonomic dysfunction in PD in Indian population. The study aimed to evaluate cardiac autonomic activity in Parkinson's disease patients with HRV parameters and study the prevalence of insulin resistance in Parkinsonism and correlate the findings with duration of Parkinson's disease.

MATERIALS AND METHODS: We evaluated autonomic function in 30 diagnosed patients of Parkinson's disease (age 40-70 years) and 30 healthy age & sex matched controls by Resting Heart Rate Varaibility and evaluated insulin resistance by measuring serum insulin levels with ACCUBIND Insulin test system. The results were analysed with SPSS version 17. Both the results were correlated with each other and with the duration of disease.

RESULTS: Statistically significant reduced HRV and both sympathetic and Parasympathetic failure have been found in Parkinson's disease patients when compared to age and sex matched controls. The prevalence of insulin resistance is also significantly high in Parkinson's disease. A significant negative correlation was found between the LF (ms2),HF (ms2) values and duration of disease in this study.

CONCLUSION: Abnormalities of autonomic function and prevalence of insulin resistance were integral and present across all the stages of PD patients which may be associated with the adverse cardiac events. Early recognition and treatment of these may decrease morbidity and improve quality of life of PD patients.

KEYWORDS: Autonomic dysfunction, Parkinson's disease, Heart Rate Variability, Insulin resistance.

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ABBREVIATIONS

ANS : AUTONOMIC NERVOUS SYSTEM

AR : AUTO REGRESSIVE

BMI : BODY MASS INDEX

BP : BLOOD PRESSURE

DBP : DIASTOLIC BLOOD PRESSURE

ECG : ELECTRO CARDIO GRAM

ELISA : ENZYME LINKED IMMUNO SORBENT ASSAY

FFT : FAST FOURIER TRANSFORM

HF : HIGH FREQUENCY

HR : HEART RATE

HRV : HEART RATE VARIABILITY

IR : INSULIN RESISTANCE

LF : LOW FREQUENCY

MI : MYOCARDIAL INFARCTION

NIBP : NON INVASIVE BLOOD PRESSURE

PD : PARKINSON'S DISEASE

PNS : PARASYMPATHETIC NERVOUS SYSTEM

REM : RAPID EYE MOVEMENT

SD : STANDARD DEVIATION

SNS : SYMPATHETIC NERVOUS SYSTEM

ULF : ULTRA LOW FREQUENCY

VLF : VERY LOW FREQUENCY

1.INTRODUCTION

"A good stance and posture reflect a proper state of mind"

-MORIHEI VESHIBA

"Involuntary tremulous motion, with lessened
Muscle power, in part not in action and even
Supported, with a propensity to bend forward and
to pass from a walking to running pace, the senses and
intellects being uninjured"

- JAMES PARKINSON

Therefore, Tremor, rigidity coupled with bradykinesia are the characteristic features of Parkinson's disease which is the one of the common neurodegenerative illness affecting the old age of the world. It affects both men and women. The average age of onset is about 60 years. But cases have been reported even at late twenties.

The incidence rate of Parkinson's disease worldwide varies from 4.9 to 26 per 100000. (Mac Donald et al 2000; Rajput AH et al 1984) [1&2] The incidence of Parkinson's disease increases with ageing. It is estimated that prevalence will increase dramatically in future

decades. Henceforth, the world is in a critical position to find a disease modifying strategy for Parkinson's disease to alleviate the problem of increasing prevalence.

Patient can be considered to have Idiopathic Parkinson disease when they have bradykinesia and at least one of the following signs : rigidity, tremor, gait disturbances or postural instability with no other known causes. (Daniel and Lees 1993).[54]

The clinical features like resting tremor, rigidity, bradykinesia and gait impairment are considered to be dopaminergic features. Additional clinical features such as speech difficulty, autonomic disturbances, mood disorders, sleep dysfunction, cognitive impairment and dementia are considered to be non-dopaminergic features.

The neuropathology of Parkinson's disease is found to be degeneration of dopamine producing and secreting nerve cells in the extrapyramidal brain structures and deposition of α-synuclein in the nerve cells. It also affects many regions of brain apart from basal ganglia and the neuropathology of Parkinsonism also affects the central and peripheral autonomic nervous system. (Baraak H et al 2003, Blaszczy JW et al 1998, Lozza A et al 1997)[4,5,6]

In addition to that the disease also extends into the peripheral autonomic nervous system involving sympathetic ganglia, cardiac sympathetic afferents and the myenteric plexus of the gut. (Pospisil et al 2008, Jankovic J et al 2008) [7,8] Recent studies suggest that the non-dopaminergic clinical features could precede the imminent clinical manifestations of Parkinsonism. (Santa Maria J et al 1986, Ponsen MM et al 2004,5,6).[9,10]

A healthy human heart doesn't beat at regular rate. It shows variation from one beat to the next. The heart rate is being greatly influenced by external and internal stimuli and regulated by autonomic nervous system. This variation of heart rate between the successive beat is called as Heart Rate Variability which bears a close relationship to sympathetic and parasympathetic nervous system. Heart rate variability indices are utilized worldwide for the assessment of cardiovascular risk in individuals including bradyarrhythmia, tachyarrhythmia, angina, myocardial infarction and hypertension.

Heart rate variability is a non – invasive and very useful tool in detecting cardiovascular autonomic disturbances. Autonomic dysfunction becomes one of the cardinal clinical feature of Parkinson disease including the manifestations like perturbation in cardiovascular

regulation, orthostatic hypotension, hyperhydrosis, defects in pseudomotor function etc . (Valko PO et al 2012, Trachani E et al 2012, Sauvageot N et al)[11,12,13] Many Parkinsonism disease patients show autonomic disturbances during the early course of illness in addition to motor symptoms. Changes in the cardiovascular disturbances can be easily detected by HRV indices even in the early phase of disease. Early detection and management of sympatho –vagal imbalance using HRV indices in time and frequency domain reduces the mortality rate in Parkinson's patients.

Thus HRV is a non-invasive tool not only for research but also for early detection of complication and timely management in Parkinsonism disease patients. (Pursiainen et al 2002)[14]

Insulin, a peptide hormone secreted by beta cells of pancreas regulates the body response to store energy after a meal. Serum insulin concentration increases in a direct proportion to glucose concentration. Aminoacids and ketoacids can also stimulate its secretion. (Ganong 22nd edition)[69] Moreover, the secretion of insulin is under the neuronal control, and the anticipation of meal or thought of food stimulate insulin secretion without the presence of glucose by activating the parasympathetic pathway.

The brain was thought to be an insulin independent organ for a longtime. (Healy DG et al 2004)[149] But, insulin receptors are richly expressed throughout the brain in neuronal and glial cells which suggest that a role of insulin in cerebral function. (Abbott MA et al 1999, E.Ferrannini et al 1999, R.J. Schuling kamp et al 2000) [150,151,152] Thus, Insulin an ancient hormone with its novel role is vital for proper functioning of brain and it seems to play an essential role in the regulation of neuronal proliferation, apoptosis, synaptic transmission and neuronal degeneration .

Eventhough insulin and insulin receptors are present throughout the brain, they are densely present in hippocampus, hypothalamus, basal ganglia and frontal lobe. Chronic hyperinsulinemia and insulin resistance or reduced insulin sensitivity may have a negative influence on neurons. Many studies have revealed the fact that insulin resistance is highly prevalent in Parkinsonism disease patients. (Craft & Watson, 2004).[17]

In Parkinson's disease, high prevalence of abnormal glucose tolerance, chronic hyperglycemia, abnormal insulin levels and insulin resistance potentiates the progression of neurodegeneration which in turn accelerates autonomic dysfunction, motor and cognitive impairment. (Boyd et al 1979).[16]

Animal studies have thrown light to the hypothesis that there is an essential role for insulin to regulate the functions of dopamininergic nerve cells of brain. (Craft & Watson 2004) [17]Chronic hyperglycemia and hyperinsulinemia resulting from insulin resistance ends in oxidative stress and the production of oxygen free radicals out of the above mechanism may potentiate the degeneration of dopaminergic nerve cells. (Saller & Chiodo 1980, Stanahan et al 2008).[18,19]

Thus, chronic hyperglycemia, hyperinsulinemia becomes one of the risk producing agent for the onset at early age and rapid progression of Parkinson's disease. Since the association between the insulin resistance and neurodegeneration have become evident recently, knowledge of the specific mechanisms relating insulin dysregulation and neurodegenerative diseases may allow the development of drugs to decelerate neurodegeneration in future.

Therefore, current study was taken up with the aim of assessing the cardiac autonomic activity in patients with Parkinsonism disease by analysing HRV parameters and to estimate the insulin resistance by evaluating serum insulin levels and as compared with normal controls.

2. REVIEW OF LITERATURE

Parkinson's disease is the commonest disabling neurodegenerative illness affecting the old age of the world's population, occurs in all ethnic groups and all socio economic classes which is characterised by impairment of voluntary movement, rigidity, tremor. It has been called "Happy disease" because of the characteristic stooped posture and chaplinesque shuffling.(Kelly 1995) Parkinson's disease is increasingly common disease of elderly patients, increasing the morbidity of old age and imposing challenge to the Physician community. Information on the pathophysiology and treatment strategies of Parkinson's disease are so voluminous. This review offers a glimpse into the rich history regarding Parkinson's disease.

Since the current study is concerned with the changes in HRV parameters and autonomic dysfunction brought about by Parkinson's disease and prevalence of insulin resistance in Parkinson's disease patients by evaluating serum insulin levels, the literature pertaining to Parkinson's disease, abnormalities in HRV and Insulin resistance alone are reviewed.

2.1. PARKINSON'S DISEASE

The clinical features resembling those of Parkinsonian illness has been explained in several study sources like an Egyptian Papyrus, an ayurvedic medical treatise, the Bible and Galen's writings. There are no references related to Parkinson's disease after Galen until the 17th century. Several authors including Sylvius, Gaubius, Hunter and Chromel described about the elements of disease in 17th and 18th century. (Garcia Ruiz PJ 2004, Lansk JK 2010, Koehler PJ 1997)[153,154,155] James Parkinson, English Physician explained the clinical features of disease as Paralysis Agitans in his book "An Essay on the Shaking Palsy".

In 1881, contributions by Jean Martin Charcoat were a milestone in the understanding of disease and he renamed the disease as Parkinson's disease in honour of James Parkinson. Lewy body which is a pathognomonic of Parkinson's disease was first described by Frederic Lewy in 1912. Studies of Arvid Carrison and Oleh homykiewicz on neurotransmitter dopamine revealed the underlying biochemical changes of Parkinson's disease in 1950.

Anti cholinergic drugs and surgery (lesioning of the corticospinal pathway and basal ganglia) were the only treatment for Parkinsonian illness till the discovery of Levodopa by Casimlin Funk. Deep brain

stimulation entered the clinical practice as a possible treatment by the late 1980s.The main component of Lewy body was found to be α-synuclein in 1997.

2.2. CLASSIFICATION

Parkinson's disease belongs to G-20, 21, 22, 23 of ICD-10.[22]

This classification was approved by the International Conference for the 10th revision of the International Classification of Disease in 1989 adopted by the forty third world Health Assembly.[22]

2.3. EPIDEMIOLOGY

Parkinsonism is the one of the common neurodegenerative illness disabling old age of the community. It affects several million people of the population of the world. It affects 0.6% of people living in the industrialized countries. The Prevalence rate of Parkinson's disease ranges between 18 to 418 per1 lakh . (Zhang Zx, Roman GC 1993)[23] The Prevalence rate of Parkinson's is more common in the elderly and it is rare before 50 years of age. The Prevalence of Parkinson's disease increases 2 times in those over 60 years of age and 10 times in the population over 80 years.

A community based study performed on epidemiology of Parkinson's disease in the Kolkota city, India found prevalence rate of Parkinsonism as 52.85/1,00,000 and incidence rate as 5.71/1,00,000 per year. (Das SK et al 2010)[156] One prospective cohort study in Italian population found a two-fold increased risk of Parkinson's disease in men compared with women. (Balderschi M, Di Carlo A et al, 2000)[24] Male predominance and rural living are closely correlated with increased Prevalence of Parkinson's disease. (Martilla RJ, Rinnie UK, 1976)[25] It is found that there will be increased risk of Parkinson's disease in persons exposed to pesticides and reduced risk with smoking. Parkinson Day has been celebrated on 11th April on the account of increasing awareness among the public. A red tulip is chosen as the symbol of the disease which represents James Parkinson, a Dutch horticulturalist.[26]

2.4. ETIOLOGY

The aetiology of Parkinson's disease remains obscure and idiopathic Parkinson's disease is common in most of the people affected. However, 10-15% of Parkinson's disease cases are inherited .(Moore DJ,West AB et al 2005; Hattori N, Mizuno Y 2004; Mcnaugt, Olanow) [27,28,29] Recent concepts have implicated environmental toxin as possible etiological agent of Parkinsonism disease in persons who have been

rendered susceptible by their genetic profile or advancing age. (Tanner CM 2003)[30] Though many toxins, occupation, microbial organisms have been reported to have an association with Parkinsonian illness, none have been proved to be an etiological agent. Recent genetic and experimental evidences have suggested that defects in ubiquitin proteosome system play a essential role in the pathogenesis of both familial and sporadic forms of Parkinsonian illness. (Petrucelli L, Deeivson TM,2004)[31]

2.5. ENVIRONMENTAL FACTORS

Several environmental factors such as MPTP (1-methyl-4-Phenyl-1,2,3,6- tetra hydro pyridine), exposure to pesticides, well water drinking and rural living have been linked to development of Parkinsonism in patients. (Tanner CM, Goldman SM 1996; Seidler A, Hellenbrand W 1996)[32,33] MPTP, a street drug contaminant can cause human Parkinsonism. Post-encephalitic Parkinsonism resulting from Endemic encephalitis lethargica sets an example of infectious disease as a causative factor of Parkinson's disease. Studies have revealed that negative association of smoking with the development of Parkinson's disease. (Gorell JM, Rybicki BA et al 1999) [34] The incidence of Parkinson's disease has been reported to be inversely related to the following: Frequency of bowel movements, coffee drinking, alcohol

consumption, hypertension, head trauma. (Ross GW,Abbott RD et al 2000; Benedetti MD, Bower JH et al 2006 ; Paganini – Hill A 2001)[36,37,38]

2.6. GENETIC FACTORS

There is strong association between increased risk of development of Parkinson's disease and positive family history especially in patients with early onset Parkinsonism. Around 10% of people with Parkinson's disease possess a first degree relative with the clinical features of Parkinsonism. About 6% of people are known to have Parkinson's disease which may occur due to mutation of one of the specific genes. (Tanner CM,Goldman SM 1996; Taylor CA,Saint-Hilaire MH et al 1999;Rybicki BA,Johnson CC et al 1999; Rocca WA,MC Donnell SK et al 2004) [32,40,41,42]

GENETIC MUTATIONS IN PARKINSON'S DISEASE

2.6.1. PARKIN MUTATIONS

Autosomal recessive juvenile Parkinsonism is found to be resulting from a mutation in the gene coding for the protein called Parkin. It is characterized by Parkinson's disease of very early age at onset of average 26 years. The mutation in the Parkin gene which is a 465AA/52 KDa protein includes deletion, multiplication and point mutation. (Matsumine

H et al 1997; Mizuno Y,Hattori N et al 2001)[44,45] The Parkin gene is diffusely expressed in the cytoplasm, nucleus, Golgi and neuronal processes. (Horowitz JM et al 2001).[46] It is not clear precisely how Parkin mutations lead to neurodegeneration. However, it likely relates to a loss of ubiquitin ligase activity and a reduced capacity to label misfolded substrate proteins for proteosomal degradation.

2.6.2. UCH-L1 MUTATIONS

There is mutation in the gene Ubiquitin C terminal Hydrolase-L1 which is associated with a familial form of Parkinsonian illness and characterized by people presenting with early age at onset about 49-51 years. It clinically resembles sporadic form of Parkinson's disease and which responds to Levo-dopa therapy effectively .(Leroy E et al 1998)[47]

2.6.3. α - SYNUCLEIN MUTATION

α -Synuclein protein is expressed throughout the central nervous system especially in pre-synaptic terminals, lipid membranes and ventricles. (Goedert M 2001) [47] α -synuclein mutation leads to a clinical picture resembling those of idiopathic Parkinsonism presenting at early age and a high occurrence of dementia.

2.6.4. DJ-1 MUTATION

Park 7 is a protein which in human is encoded by Park 7 gene also known as DJ-1. It contributes 1-2 % of early onset familial Parkinson's disease cases which is characterized by early age of onset around mid thirties, slow rate of progression, good L-Dopa response, presence of dystonia and psychiatric disturbances.

2.6.5. PINK 1 MUTATIONS

PINK 1 mutations are associated with Autosomal recessive pattern of inheritance, early age of onset of Parkinsonian disease in which patients present clinically at their 32-48 years, slow progression and good response to L-dopa.[49]

2.6.5. LRRK2 MUTATIONS

LRRK2 gene codes for a protein called Dardarin, a Basque word for tremor. An Autosomal dominant form of Parkinsonian illness has been linked with mutation in the gene coding for LRRK2 (Leucine Rich Repeat Kinase2) associated with the loss of dopaminergic nerve cells in striata nigra pars compacta and few cortical lewy bodies.

2.7. NEURONAL ORGANIZATION OF BASAL GANGLIA

Basal ganglia are the deep nuclei of the cerebrum which includes Caudate nucleus, Putamen, Globus Pallidus,substantia Nigra and subthalamic nucleus located on each side of brain. The Basal ganglia do not receive input from spinal cord. But they receive most of their input signals from the cerebral cortex. Their action on the motor area of cortex is mediated through thalamus. (Nakano K, Kayahara T et al 2000) [50]

The essential role of Basal ganglia in control of voluntary motor activity includes cognitive control of motor activity, timing and scaling of intensity of movements, subconscious execution of movements.

2.7.1. CONNECTIONS & OPERATIONS OF BASAL GANGLIA

Neurons of striatum begin to discharge before any movement which suggests that these neurons help to select the movement that is to be made. Activity in the Putamen is related to the occurrence of movement of body. Activity in caudate nucleus is related to eye movement. Most regions of cerebral cortex project topographically to striatum with the exception of primary visual and auditory cortex. (Berne & Lewy) [52]

The corticostriate projections arise from neurons in layer V of cortex. The striatum influences neurons in the ventro anterior & ventro lateral nuclei of thalamus by 2 pathways – direct and indirect. The ultimate effect of Direct pathway through the Basal ganglia to motor cortex results in enhancement of the motor activity. The overall effect of indirect pathway is to reduce the motor activity.

Dopamine is the major neurotransmitter of the neurons of substantia nigra Pars Compacta of the basal ganglia. The release of dopamine in nigrostriatal tract results in stimulatory action on direct pathway and an braking action on the indirect pathway. This is due to the action of dopamine on different types of receptors that are found in the internal segment of Globus pallidus (D1 receptors which are excitatory in nature) and in the external segment of the Globus Pallidus (D2 receptors, inhibitory in nature). The overall effect of release of dopamine in the above two scenario is a enhancement of activity in the motor areas of the brain. (Berne & Lewy)[52]

2.8. PATHOPHSIOLOGY

Parkinson's disease most commonly occurs due to idiopathic degeneration of nigrostriatal system of dopaminergic neurons. It is also found that there is greatly diminished activity of dopamine secreting

nerve cells caused by neuronal death in the Pars compacta region of substantia nigra. There is an imbalance between excitation and inhibition in the basal ganglia created by the loss of dopaminergic inhibition of the Putamen. The resulting increase in inhibitory output to the external segment of globus pallidus decreases inhibitory output from the subthalamic nucleus, and this increases the excitatory output from this nucleus to the internal segment of globus pallidus. This in turn increases the inhibitory output from this segment to thalamus, causing a reduction in excitatory drive to cerebral cortex. (Berne & Lewy; Adam & Victor) [52,53]

2.9. DIAGNOSTIC CRITERIA

Criteria from UK Parkinson's disease Brain Bank and US National Institute of neurological disorders & stroke require slowness of movement plus either one of the following signs and symptoms: rigidity, resting tremor or postural instability. Other possible causes for these symptoms have to be ruled out. (Daniel SE, Lee AJ 1993)[54]

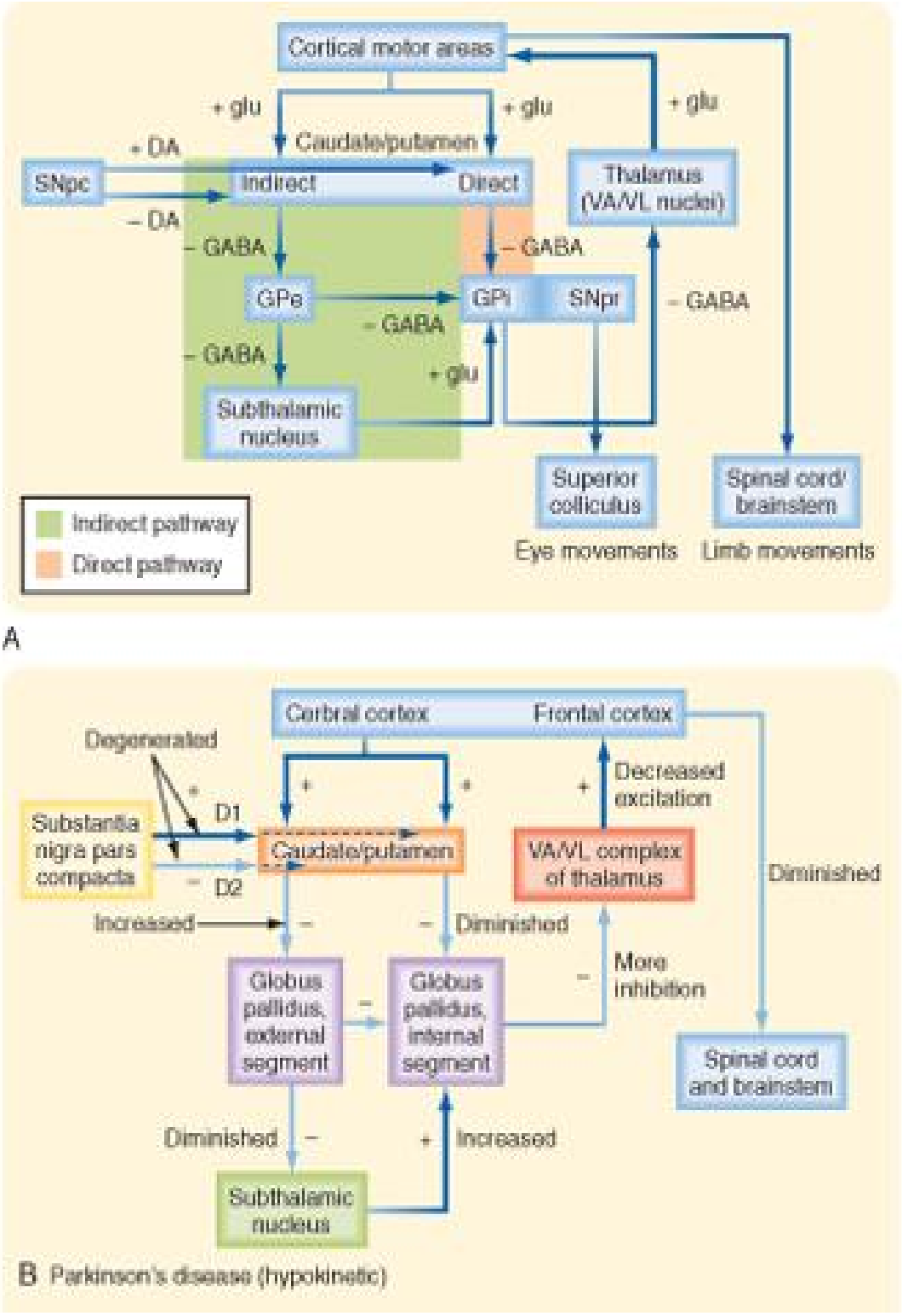


Figure 1 : NEURONAL ORGANISATION OF BASAL GANGLIA AND PATHOPHYSIOLOGY OF PARKINSON'S DISEASE

2.10. HOEHN AND YAHR SCALE

The Hoehn and Yahr scale is widely used clinical rating scale which measures the severity of motor impairment in Parkinson's disease.[92]

1: Only one sided involvement, usually with minimal or no functional disorders

2: Both sided or midline involvement without affecting the balance

3: Both sided disease: mild to moderate disability with diminished postural reflexes; physically independent

4: Severe disabling disease; still able to walk or stand without any assistance

5: Confound to bed or wheelchair unless aided

2.11. MOTOR SYMPTOMS IN PARKINSON'S DISEASE

The cardinal features of Parkinson's disease are bradykinesia, tremor and rigidity. The patient is unable to initiate the voluntary movements (akinesia) or the voluntary movements are decreased (hypokinesia). Difficulty in initiating movement is because of hypertonicity of muscles. Manifestations of bradykinesia include: delayed

motor initiative as evidenced by prolonged reaction time, slow performance of voluntary movements, mask like facies, absence of normal associated movements, shuffling or festinant type gait in which patient is bent forward and walks quickly with short steps as if trying to catch up centre of gravity or preventing himself from falling, retropulsion i.e. when a walking patients is suddenly pulled backwards, he begins to walk backwards and is unable to stop. (Adam & Victor) [53]

2.11.1. RIGIDITY

Rigidity refers to increase in tone of the muscles. It occurs due to increased tone in both the protagonist and antagonist muscles. Mainly large proximal group of muscles are affected. Usually there occurs uniform resistance to flexion giving a feeling as if lead pipe is being bent (lead pipe rigidity). Sometimes there is a series of catches during passive motion of limbs (cog wheel rigidity). It is because of rigidity, posture becomes that of flexion attitude in which back is flexed, arms are abducted and flexed and the knees are bent. In advanced cases, the rigidity may increase to such an extent that s statue –like appearance is produced with complete absence of movements. (Harrison, Adam & victor)[53,55]

2.11.2. TREMORS

Tremors, (i.e. involuntary rhythmic oscillatory movements of distal parts of limbs and head) seen in Parkinson's disease have following characteristics: The tremors are present in the resting state, but disappear on initiation of activity. It is Hallmark of Parkinson's disease and so popularly known as resting or static tremors. Frequency of tremor ranges from 4 - 6 times/sec. It is frequently seen as pill-rolling movements of the hand, i.e., rhythmic contraction of thumb over first two fingers. Tremors are suppressed during sleep and exaggerated by stress, anxiety and excitement. The tremors are observed as rhythmic movements of pronation and supination in fingers, hands, lips or tongue. The tremors seem to occur due to pacemaker activity in the nucleus ventralis intermedius of thalamus. Thalamic neurons exhibit an intrinsic auto-rhythmicity and probably get unmasked due to increase in the inhibitory input from the pallidum. (Harrison , Adam & Victor)[53,55]

2.12. NON MOTOR SYMOTOMS IN PARKINSON'S DISEASE

Non-motor clinical manifestations in Parkinson's disease includes disorders of cognitive function , sleep wake cycle, regulation of mood, function of autonomic nervous system, sensory system function and pain modulation.

In addition to dopaminergic nigrostriatal system, neuropathology of Parkinson's disease also involves the mono-aminergic neurons of locus ceruleus, dorsal vagal nucleus, hypothalamus, limbic cortex and peripheral autonomic nervous system. Hence, non motor symptoms become more prevalent and prominent during the course of disease. (Braak H, Del Tredici K et al 2003; Blaszczyk JW 1998) [4,5]

Non motor symptoms of Parkinson's disease act as early or preclinical biomarker of Parkinsonian illness in an otherwise asymptomatic individuals as evidenced from clinical studies performed to assess the olfactory dysfunction or REM sleep disturbances. (Ponsen MM, Stoffers D et al 2005; Stiasny – Koloster K, Magerl W et al 2005)[10,56]

2.12.1. SENSORY SYMPTOMS

Parkinson's disease patients experience numbness, tightening sensation, tingling sensation, diffuse pain, neuralgic pain, burning sensation. Pathophysiology underlying these sensory symptoms is probably resulting from altered central pain pathway as a part of the neurodegeneration, absence of dopaminergic pain inhibitory input to the dorsal horn synapse, absence of noradrenergic descending pain inhibitory input from the locus ceruleus to the dorsal horn of the spinal cord and

widespread cortical lewy body degeneration . (Buzas B, Max MB2004) [57,58]

2.12.2. OLFACTORY DYSFUNCTION

It has been established from a number of studies that olfactory dysfunction i.e. impairment in odour detection, discrimination, and identification is an early clinical sign in 90% of Parkinson's disease patients. (Katzenchlager R, Zijlmans J et al 1999).[59]

2.12.3. NEUROPSYCHIATRIC DYSFUNCTION

Neuropsychiatric features such as mood disorders, cognitive dysfunction and complex behavioural disorders are associated with Parkinson's disease in contrary to J.Parkinson's original descriptions about "the senses and intellect being uninjured". (Adam & Victor)[53]

2.12.4. SLEEP DISORDERS

Sleep disorders including difficulties in sleep initiation , increased number of awakenings during sleep, muscle cramps, dystonia or nocturnal motor manifestations such as difficulties in moving in bed, motor restless leg syndrome, nocturnal bladder incontinence, nocturnal mood disorders, hallucination and day time sleepiness are the most

frequent non-motor clinical manifestations in patients with Parkinsonian disease. The neurodegeneration of the Parkinson's disease affecting the sleep structure, respiratory disturbances are the various mechanisms suggested for sleep dysfunction in the Parkinson's disease patients. (Tandberg E, Larsen JP et al)[60]

2.12.5. AUTONOMIC DYSFUNCTION

Autonomic disturbances has been reported in Parkinsonism patients by James Parkinson. Clinical features of derangement of both the central and peripheral autonomic nervous system could be seen in Parkinsonism. Presence of α-synuclein within the sympathetic ganglia and antibodies to sympathetic nerve cells in Parkinsonism has been found recently. α-synuclein could be detected in the higher autonomic centres including the hypothalamus, dorsal vagal nucleus and intermediolateral nuclei of thoracic cord.Presence of Lewy bodies within the central and peripheral autonomic nervous system has been believed to be a biomarker for neuronal loss.

Autonomic dysfunction becomes the prominent universal feature of the Parkinson's disease that includes orthostatic hypotension, constipation, sialorrhea, seborrhoea, hyperhydrosis, heat intolerance, urinary and sexual dysfunction. Studies have found evidence for

cardiovascular dysfunction in 30% of cases, urogenital dysfunction in 32% of cases and gastro intestinal dysfunction in 36% of cases. (Magalhae M et al 1995) [61]

Most of the people suffering from Parkinsonism initially present with autonomic disturbances supporting the hypothesis that these autonomic disturbances might act as a predictive biomarker in Parkinsonism.

CARDIOVASCULAR AUTONOMIC DYSFUNCTION

Symptomatic orthostatic hypotension is a late feature of the Parkinsonian illness and Peripheral symptomatic cardiovascular denervation is prominent in Parkinson's disease patients. Cardiovascular Autonomic dysfunction is highly prevalent in patients with bilateral severe bradykinesia and rigidity. (Spiegel et al 1969, Martilla et al 1974, Happaniemi 2003)[62,63,64]

GASTRO INTESTINAL DYSFUNCTION

Neuropathology of Parkinsonism affecting the peripheral autonomic nervous system also affects the myenteric plexus and results in the formation of Lewy body in the colonic autonomic plexus leading to subsequent colonic sympathetic denervation. Studies have found either

constipation or increased transit time in as many as 80% of Patients with Parkinsonian illness.(Jost WH,1997)[65]

UROGENITAL DYSFUNCTION

Urogenital dysfunction such as difficulties in erection , difficulty in ejaculation, increased frequency of urine, urgency of micturition, incomplete bladder evacuation, double urination and urging incontinence becomes the late feature of Parkinson's disease.

2.13. HEART RATE VARIABILITY

Heart Rate Variability represents the variability of both consecutive heart rate and consecutive RR intervals. The other terms of Heart Rate Variability used are cycle length variability, Heart period variability, RR interval variability, RR interval tachogram. The regulatory mechanisms of Heart Rate Variability takes its origin from sympathetic and parasympathetic nervous system and thus Heart Rate Variability can be used as quantitative bio marker of autonomic nervous system. (Task Force 1996)[66] Stress, certain cardio vascuar diseases, and other pathological conditions have a profound influence on HRV. (Berntson,1999) [67,68]

2.13.1. AUTONOMIC NERVOUS SYSTEM

Langley, an English Physiologist who first coined the term "AUTONOMIC NERVOUS SYSTEM" in 1898 to describe the part of nervous system which deals with the functions occurring automatically without the knowledge of Individual. The Autonomic Nervous System controls the functions of the involuntary organs of the body such as heart, blood vessels, exocrine and endocrine glands and all visceral organs. The Autonomic Nervous System responds to endogenous and external stimuli and a constant internal environment (Homeostasis) is maintained by Autonomic Nervous System. It regulates visceral activities, coordinates body's response to stress and regulates the endocrine system. (Ganong 2012)[69]

Based on their anatomic, functional and neurochemical properties, the two divisions of Autonomic Nervous System are the Sympathetic and Parasympathetic Nervous System. The Parasympathetic and Sympathetic divisions are inturn divided into Preganglionic and Postganglionic neurons.

The Parasympathetic preganglionic neurons have their cell bodies in the brainstem and in the II, III, IV sacral segment of spinal cord. Their axons come out of the CNS via the cranial and sacral spinal nerves and

synapse with postganglionic neurons in the specialized ganglia close to the visceral organ. The vagus nerve originating from the dorsal vagal nuclei and the nucleus ambigus in the medulla oblongata carries the most widespread cranial parasympathetic output.(Loewy and Spyer 1990)[70]

The cell bodies of sympathetic preganglionic neurons are located in the intermediolateral horn of thoracolumbar segment of the spinal cord. Their axons reach the Para vertebral ganglia of the sympathetic trunk and synapse with the post ganglionic neurons.

Almost all the organs of our body are innervated by both sympathetic and Parasympathetic nerves. These sympathetic and parasympathetic nerves are usually acting in a reciprocal manner. But, in some organs the sympathetic and parasympathetic nerves act synergistically to regulate the visceral function. (Benorroch 1997, Crick et al 2000)[72,73]

The neurotransmitter in all parasympathetic neurons is Acetylcholine. Nor adrenaline is the primary neurotransmitter in sympathetic postganglionic fibres exceptions are those cholinergic fibres innervating the sweat glands and some cholinergic vasodilator fibres. (Willis WD 1993, Benarroch 1997)[71,72]

The frontal lobe cortex, the limbic cortex, the amygdala and the hypothalamus are the main CNS structures controlling the Autonomic Nervous System. (Willis 1993) [71] The highest level of integration of Autonomic Nervous System function appears to be the frontal lobe cortex. The limbic system regulates autonomic response to emotional and affective expressions. The hypothalamus serves as the most important regulator of Autonomic Nervous system that integrates the cortical autonomic structures of the preganglionic autonomic neurons.

2.13.2. CARDIAC AUTONOMIC ACTIVITY

The Autonomic Nervous system regulates many of the functions of cardiovascular system through its two branches sympathetic and parasympathetic. Both heart and vascular system are innervated by sympathetic and parasympathetic nerves. The parasympathetic nerve (vagus) supplies SA node and conduction system of heart. Sympathetic nerves mainly innervate the cardiac muscle and vascular system. The sympathetic branch of Autonomic Nervous system increases the cardiac action and the parasympathetic branch of Autonomic Nervous system slows the cardiac action. (Benarch 1997, Crick et al 2000) [72,73]

The autonomic nerve supply of heart is controlled by cardiac autonomic centres situated in the medulla which form the integrating

centre of cardiac autonomic reflex. Rostral ventral Lateral Medulla also known as vasomotor centre regulates the cardiac sympathetic nerves and excites the heart. The cervical sympathetic nerves form the efferent limb of cardiac autonomic reflex.

The nucleus ambiguus also called as medullary parasympathetic centre or cardiac vagal centre receives afferent from various peripheral organs and in turn sends inhibitory output via vagus nerves to decrease the heart rate and force of contraction of heart. Some inputs exciting the cardiac sympathetic centre will inhibit the parasympathetic centre. There is an antagonist effect of these both vital centres on each other. (Berne &Lewy)[52]

When the vagus nerve is stimulated, there will be release of Acetylcholine into all of the organs except the adrenal glands. The acetylcholine released into SA node results in decrease in heart rate through the opening of channels in the cell membrane that are permeable to K^+ but not Ca^{2+}. So, K^+ moves out of the cell without this inward flow of Ca^{2+} and postsynaptic cell is inhibited which slows down the Heart rate. (Burnstock 1986, Sakman B, Noma et al1983)[75]

Heart has its own inherent activity generating its own impulse at constant rhythmic intervals which is seen as spontaneous firing of

Pacemaker cells in the wall of Right atrium. These Pacemaker cells are small round cells with few organelles that are connected by large number of gap junctions. (Ganong 2012)[69]

The autonomic inputs of heart are mediated by SA node and AV node which are responsible for cardiac rhythms. SA node being the primary Pacemaker of heart, initiates an electrical signals which in turn begins the pumping action of heart. Then, the signal passes through the AV node which spreads the electric signals throughout the ventricles of heart by specialised conduction pathway.

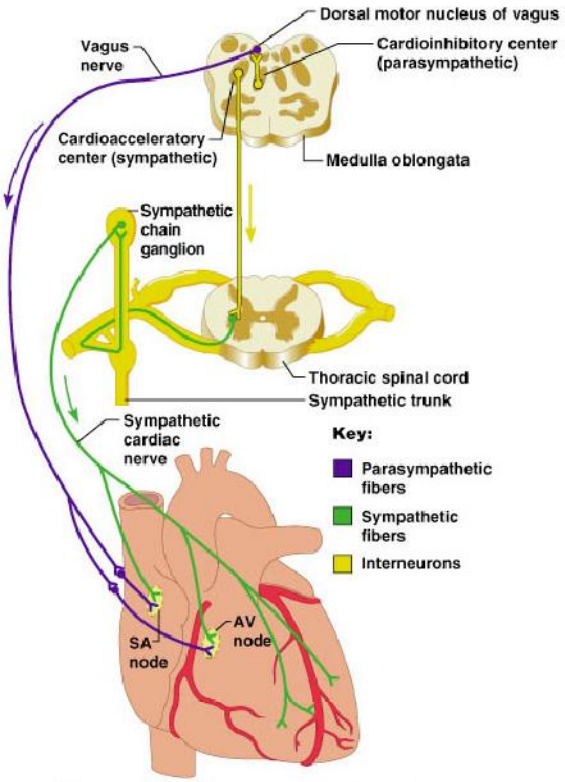


Figure 2 : INNERVATION OF HEART

The intrinsic firing rate of uninnervated SA node is 100 beats per minute. (Ganong 2012)[69] The sympathetic system increase the rate of depolarisation of SA node in the innervated heart, while the parasympathetic system through the vagus nerve decreases the firing rate of SA node. The net accelerating and decelerating effects of the cardiac sympathetic and parasympathetic system will determine the cardiac sympathetic and parasympathetic tone respectively.

The resting vagal tone dominates over the sympathetic tone that makes the heart rate below 100. The heart rate is conventionally measured by number of beats per minute. The duration of cardiac cycle of all heart beats occurring in one minute, even under resting condition is not the same. There is beat to beat variability of R-R interval in milliseconds. This spontaneous beat to beat variation is known as Heart Rate Variability. (Task Force 1996)[66]

The physiological origin of HRV is the fluctuations of activity of cardiovascular vasoconstrictory and vasodilatory centres in brain. Usually the fluctuations are due to baroreflex mediated BP oscillations, circadian rhythm and thermoregulation. All these factors influence the length of R-R interval. The original balance between their activities keep on

changing constantly in order to achieve maximum considering of all internal and external stimuli. (Task Force 1996)[66]

Higher the HRV represents the optimal balance between the sympathetic and parasympathetic nervous system. The higher the HRV, the quicker the heart adapts to external and internal stimuli and better the individual react to all these stimuli.

Depressed HRV primarily means that Heart rate is monotonously regular which indicates the depressed activity of Autonomic Nervous system to cope up with the internal and external stress. A low HRV represents a diminished ability to external environment and may suggest serious health problem. HRV is a measure of the Respiratory Sinus Arrhythmia (i.e.) the heart rate increases during inspiration and the heart rate decrease during expiration. This normal phenomenon decreases with age and also under stressful conditions.(Wheeler&Whatkins 1973, Kahna & Gik 1975, Eckeberg 1983) [76,77,78]

The Respiratory sinus arrhythmia is recorded as fluctuations in the RR intervals within the frequency range 0.15-0.4 Hz which are considered to be mediated mainly by vagus. The Heart Rate Variability is found to be the quantitative biomarker of parasympathetic function from the previous animal studies and human studies using atropine also shows

that Heart Rate Variability is a marker of Parasympathetic function.(Broutia and Nowak,1939; Berntson et al 1994)[68,79]

Heart Rate Variability can easily detect changes in body stress, while other physiological parameters are still in "normal" accepted range. Some Heart Rate Variability changes may act as a first sign of distress which reflect involvement of energy dependent sympathetic system.(Task Force 1996)[66]

The parasympathetic (vagal) influence is dominant and heart rate variations depend on vagal modulation; however, the sympathetic and parasympathetic branches continuously interact. The SA node is rich in acetyl cholinesterase, the enzyme breaks down acetylcholine which allows for rapid but brief effects from the parasympathetic activation.

Acetylcholine is released from the vagus nerve and binds with the muscarinic receptors on the SA node cells.(Ganong 2012) [69] Sympathetic activation also releases Acetylcholine in the preganglionic fibres, but most of the sympathetic postganglions, including the adrenal medulla, release epinephrine and nor epinephrine. Nor Epinephrine binds to beta-1 receptors and increases the heart rate by increasing Ca^{2+} permeability. The parasympathetic response is faster than sympathetic, likely because stimulation of the muscarinic receptors reduces the amount of nor

epinephrine released during sympathetic activation and also due to a cholinergic withdrawal of the adrenergic stimulus. (Task Force 1996) [66]

In a healthy person, autonomic tone, allow for a constant level of balance and a great range of control and adjustment of heart rate. Some adjustments in the heart rate can be made to respond to demands of active tissues in the body. In a person with good vagal tone, epinephrine and nor epinephrine are almost continuously released in small amounts, even at rest.

Since the parasympathetic division is dominant at rest, the heart rate is controlled and a balance between the parasympathetic and sympathetic stimulation is negotiated. Several studies have concluded that an increased activity of sympathetic and decreased vagal modulation increase the risk of ventricular tachycardia, ventricular fibrillation and sudden cardiac death.(Martins 1985; Pozzati et al 1996; Schwartz et al 1992) [80,81,82]

2.13.3. HRV AS A AUTONOMIC FUNCTION TESTING TOOL

The evaluation of autonomic disorders is more widely readily available with evolution and validation of reliable non invasive techniques. The techniques used to study the autonomic functions are

described of which cardio-vagal, adrenergic and pseudomotor function are most common methods involved.

The most commonly used test of autonomic function depends on changes in heart rate, blood pressure in response to breathing and to posture changes. These tests are simple, non invasive, easy to perform and are both sensitive as well as specific. The fact that variability of heart rate occurs because of autonomic innervations of SA node has been exploited to develop tests that stimulate this autonomic supply and results in variability of heart rate.

2.13.4. HEART RATE VARIABILITY TEST

There has been no quantitative marker for cardiovascular autonomic functions. Studies in last 30 years have shown a great association between Autonomic nervous system (ANS) and cardiovascular morbidity, including sudden death in arrhythmias .(Lewy et al 1994)[83]

Heart rate variability represents the hall mark of such markers. Automated measurement of Heart Rate Variability (HRV) is possible with the use of many commercial devices nowadays. But the precise measurement and assessment of HRV analysis being more complex,

European society of cardiology and North American society of pacing and electrophysiology have contributed a Task Force to appropriate standards. The guide lines and recommendations of Task Force 96 are followed in this study. "Heart Rate Variability" has been the universally accepted term to explain the variation of both consecutive heart rate and R-R intervals, which emphasizing the fact that it is the period of time between two consecutive beats that is being analyzed rather than the heart rate per second.(Task Force ,1996) [66] Heart Rate Variability is a non-invasive electrocardiographic marker reflecting the action of sympathetic and vagal component of Autonomic Nervous system on heart. It reflects the total amount of variations of both instantaneous heart rate and RR interval.

The clinical use of HRV was well recognized when **Hon and Lee** in **1965** found that, alteration in HRV precedes fetal distress before any significant variation occurred in heart rate itself.[86]

During **1985 Ewing et al** performed simple test for short term R-R difference in the detection of diabetic neuropathy.[86] **Akselrod et al (1981)** was the first person to explain about power spectral analysis of heart rate fluctuation for quantitative evaluation.[85]

Juan sztajzel et al 2004, noted that among the various available non invasive techniques to assess the sympathovagal balance, heart rate variability has evolved as a simple non invasive tool to evaluate autonomic status at sinoatrial node.[87]

Sayers et al (1973) noticed alteration on existence of physiological rhythm imbedded in beat to beat heart rate signals.[88] **Stein P.K. et al (1994)** proposed that the heart rate variability is a non invasive electrocardiographic marker which represents the activity of sympathetic and parasymapathetic component of ANS on sino atrial node of the heart.[89] **Persson et al (1983)** computed that the R-R interval variation (RRIV), as assessment of the heart rate variability and one of the simplest and most reliable test used for the evaluation of the PNS autonomic functions of the heart.[90]

Heart Rate Variability (HRV) is mediated primarily by at least three primary mechanisms:

- Vagal feedback from pulmonary stretch receptors
- Central medullary coupling between pulmonary and cardiovagal neurons
- Arterial baroreflex induced oscillations.

The resting Heart Rate Variability which comes under the grouped cardiac autonomic function tests which evaluate the cardiac division of the ANS i.e both sympathetic and parasympathetic in resting state.

2.13.5. COMPONENTS OF HRV

Beat to beat variability present even in resting condition. Parasympathetic tone dominates in the resting state and the vagal modulation primarily influences the variation in heart rate period greatly. There is a constant interaction of vagal and sympathetic activity.

The R-R interval variation existing in the resting state represents optimal tuning of the beat to beat control mechanism.(Askerold et al 1985; Saul JP et al 1990) [88,91,97]

There is excitation of vagal afferent activity and inhibition of sympathetic efferent activity resulting from vagal afferent modulation. The stimulation of sympathetic afferent activity mediates the opposite reflex effects. (Schwartz et al 1973; Malliani1982)[82,96] Efferent vagal activity is believed to be tonic restraint by cardiac afferent sympathetic activity. The both sympathetic and vagal efferent activities directed to sino atrial node are best explained by discharge greatly synchronous with each cardiac cycle that can be modulated by central autonomic centres and respiratory centers and peripheral oscillators (fluctuations in arterial

pressure and respiratory movements).(Malliani et al 1991) [96] All these oscillations results in generation of rhythmic fluctuations in efferent neural discharge which is seen as short and long term oscillations in the cardiac cycle.

Evaluation of these rhythms influences on the state and function of (Task force, 1996).

The central autonomic Oscillators

- The sympathetic and parasympathetic efferent activity
- Humoral factors
- Sino atrial node.

2.13.6. CLINICAL APPLICATIONS OF HRV

HRV has been a non invasive test for assessing the autonomic functions in various clinical conditions, including diabetes mellitus, hypertension, angina pectoris, cardiac failure and recently used as a screening tool in patients with obstructive sleep apnea. Altered HRV is a warning sign of diabetic neuropathy and precedes the clinical manifestations. It predicts the mortality risk after MI, arrhythmias. It can be used for exercise training in the field of sports physiology. (Task Force 1996)[66]

2.13.7. CURRENT LIMITATIONS OF HRV

Inspite of the the large number of research studies, the measurement of HRV is still used in research fields and not routinely used in therapeutics. It may be due to non- availability of standard values, cost of equipment and software, variability of HRV parameters with age, gender, drug interferences and concomitant diseases.(Task Force,1996) [66]

2.13.8. HEART RATE VARIABILITY INDICES IN PARKINSON'S DISEASE:

M.kalio, T.Happaniemi et al (2000) studied cardiovascular autonomic reflexes and Resting Heart rate variability parameters in 50 patients with untreated Parkinsonism and compared with 55 normal controls to study the cardiovascular changes as a predictive marker of autonomic nervous system dysfunction in patients with untreated Parkinsonism illness. They found HF and mildly the LF were decreased and both sympathetic and Para symapathetic hypofunction has been reported. They reported significantly diminished HRV, significantly reduced BP reaction to tilting in their study. The patients presenting with hypokinesia/rigidity have much reduced HRV than patients presenting with tremor was revealed from their study.[100]

Goldstein DS, Holmes C et al (1997) found an attenuation in the myocardial uptake of marker on Positron Emission Tomography favouring a loss of myocardial sympathetic nerve terminals in patients with Parkinsonism illness presenting with autonomic failure.[101]

Hakusui et al (1994) & Orimo et al (1999) found out a diminished MIBG uptake in most of the Parkinsonism patients even in the early phase of illness using meta 123 I Iodobenzyl guanidine myocardial scintigraphy which indicates cardiac sympathetic nerve disturbances.[102]

Samay Jain, Greg J et al (2011) studied autonomic dysfunction in pupillary and cardiovascular system in Parkinsonism. They evaluated spontaneous variations of diameter of pupil in darkness, constriction and reduction velocities and HRV and cardiovascular autonomic test in 35 study subjects (17 Patients, 18 healty subjects). The results of Pupillary unrest and orthostatic standing test were highly significant in PD patients and reduced HRV was recorded in PD patients.[103]

Popsil P, Konecwy l et al (2008) studied autonomic dysfunction and progression of Parkinson's disease in 25 patients using power spectral HRV analysis which is coupled with metronome controlled breathing in lying down position .They found out significantly reduced

LF(Power),HF(power),Total Power in Parkinson's disease patients. They suggested short term HRV examination is also sensitive enough to compare the autonomic dysfunction in various stages of Parkinson's disease .[104]

Devo's D, Krumova M et al(2003) studied correlation between HRV and severity of Parkinsonism in 30 patients. They found out reduced LF(power), HF(power),PNN50 in Parkinson's disease patients.[105]

Turkka et al (1987) revealed that low levels of serum Nor epinephrine and its metabolite in Parkinsonism patients in response to standing up.[106]

Nicolas Sauvageot,Michel, Valliant et al (2007) studied HRV in 35 patients and 35 subjects and their result was reduced sympathetically induced HRV and low LF/HF ratio during sleep in Parkinsonism patients and they added a note on Postganglionic noradrenergic cardioselective denervation in Parkinsonism.

T.szili-Toro K, L.Rudas, G.Dibo et al (1999) did HRV and cardiovascular autonomic reflex test in 20 Parkinson disease patients and 18 healthy subjects. He divided the Parkinson's disease patient into

normal baroreceptor sensitivity group and impaired baroreceptor sensitivity group. Average RR interval length was shorter in Parkinson's disease patients , RMSSD was significantly lower and much reduced TP,LF,HF in Parkinson's disease patients. [107]

T.H.Happaniemi, V.Pursiainen et al(2001) have performed HRV analysis of 24 hr ECG recording in 54 untreated Parkinson's disease patients and 47 age matched normal subjects. They found low values of SDNN,VLF,LF,HF in Parkinson's disease patients.[108]

Santigo, Perez-Lioret, Maria Veronica et al (2013) suggested that orthostatic hypotension in Parkinson's disease represent cardiac autonomic dysfunction and it is predominantly of altered baroreflex activity resulting from cardiac and vascular sympathetic denervation .[109]

Shin- Yuan Chen, Cheryl C.H Yang et al (2010) did a retrospective cohort study in 24 men, 4 women with advanced Parkinson's disease patients to find out the correlation of HRV with clinical outcome in Parkinsonism patients after subthalamic deep brain stimulation. Patients underwent Deep brain stimulation for 9-32 months, day time ECG for 5 minutes. They found increase in LF power but not in HR,LF%,HF% after deep brain stimulation.[110]

Appenzeller & Goss et al (1971), Turkka et al (1987,1997), Haapaniemi et al (2000) revealed that Heart rate responses to Valasalva manoeuvre and deep breathing test is being reported as significantly diminished in patients with Parkinsonism.[111]

The BP response to isometric handgrip test is also reduced with reference to **Gross et al (1972),Van Dijik et al (1993).**[117,118] Diminished spectral power values is being obtained from 24 hr ECG recordings according to **Mastrocola et al (1999)**.[118]

Wang SJ ,Shan DE et al studied Symapthetic Skin Response(SSR) and R-R interval variation in 62 Parkinson's disease patients and 62 age matched controls. They suggested that abnormal SSR in Parkinson's disease patients is associated with the duration of illness and it may be due to intermediolateral coloumn dysfunction. The abnormal RRIV was not related to staging and duration of disease.[119]

Hirashima F, Yokota T et al (1996) evaluated 83 patients with Parkinson's disease using Sympathetic Skin Response(SSR) and sweat response to acetyl choline injection. They found significantly attenuated amplitude and abnormal SSR in Parkinson's disease. The sweat volume was low and diminished number of sweat glands had been revealed from

local sweat response to acetyl choline which reflects the dysfunction of postganglionic sympathetic fibre in Parkinson's disease.[120]

Thus, the above clinical studies have observed differences with sympathetic nervous system and parasympathetic system activities in Parkinson's disease. However differences in sample size, age of the subjects, stress conditions, stage of the disease and psychological parameters may explain the varied results by these studies.

The current study proceeds with assessment of the autonomic functions using Heart Rate Variability at rest in Parkinson disease patients with no gender preferences.

2.14. INSULIN

It is a polypeptide hormone secreted by the beta cells of islets of langerhans of pancreas concerned with metabolism of carbohydrate, protein and fats.

2.14.1. STRUTURE & BIOSYNTHESIS

The human insulin consists of 2 straight peptide chains, A (having 21 amino acid) and B (having 30 amino acid). These chains are connected to each other by two inter chain disulphide linkages A7 to B7

and A20 to B19. In addition there is an intra chain disulphide bridge between 6 and 11 amino acid of A chain. Molecular weight of human insulin is 6000 daltons.

The insulin gene is localised on chromosome 11 directs the production of pre-pro-insulin (108 amino acid, molecular weight 11,500 dalton). Pre-pro-insulin is cleaved to form pro insulin having 86 amino acid and molecular weight 9000 daltons. As the pro-insulin containing the A & B chain of insulin and the connecting peptide is guided to Golgi apparatus, disulphide linkages are established to yield the folded pro-insulin molecule. Pro-insulin is further cleaved in Golgi apparatus to form the active hormone insulin and a connecting peptide (C peptide).(Steiner DF et al2001)[122]

2.14.2. MECHANISM OF INSULIN SECRETION

Blood glucose is the major stimulant of insulin secretion. Glucose diffuses into the beta cells by binding with the GLUT2 receptors on the beta cell membrane. Inside the beta cells, glucose is oxidized to ATP by the enzyme Glucokinase which act as a fundamental glucose sensor.

Increased ATP levels results in closure of ATP sensitive K^+ channels and suppresses the K^+ efflux and suppresses the K^+ efflux

causing depolarization of beta cells. Depolarization opens voltage regulated ca^{2+} channels leading to increase in intracellular Ca^{2+}. Elevated Ca^{2+} concentration activates the mean for insulin secretion from the secretory granules by exocytosis. (Langin D et al 2001) [52]

2.14.3 REGULATION OF INSULIN SECRETION

Glucose, amino acids, free fatty acids, ketoacids, potassium control insulin secretion by a feedback mechanism. Gastrointestinal hormone increases insulin secretion. Growth hormone, cortisol, glucagon also stimulate insulin secretion. But, prolonged secretion of these hormones leads to burning out of the islets resulting in Diabetes mellitus. Sympathetic nerves and epinephrine inhibit insulin secretion. Parasympathetic nerves and acetylcholine stimulate insulin secretion. (Berne & Lewy) [52]

2.14.4. PLASMA LEVELS AND FATE OF INSULIN

Average peripheral plasma insulin level is 10 μu/ml. Insulin circulates unbound to any carrier protein. Half life of insulin in the plasma is 5 to 8 minutes. Insulinase, a protease enzyme in the kidney and liver degrades insulin.

2.14.5. INSULIN RECEPTOR

It is a protein kinase receptor which contains enzyme activity. About 2 to 3 lakh insulin receptors are present in the cell membrane of target tissue for insulin.(Kido Y et al 2001; Seinos,Seino M,Bella I 1990) [123,124,125] Insulin receptor is a tetramer having 2 α subunits and 2 β subunits. One α subunit is bound with β subunit by a disulphide bond to form a dimer. Two such identical dimers are located on the outer surface of the plasma membrane and contain the insulin binding domain. B subunit spans across the plasma membrane and reside largely within the cytoplasm. These have tyrosine kinase domain.

Down regulation of insulin receptors results in Insulin resistance. Among the insulin receptor isoforms, insulin receptor –A isoform is mainly seen in nerves and mediates neuological functions such as synaptic transmission, plasticity. The effects of Insulin receptor –B isoform are typically metabolic.(Belfiore A et al 2009) [125]

2.14.6. MECHANISM OF INSULIN ACTION

The first and overall rate limiting step in insulin action is transport of insulin through the capillary wall to the target tissues such as muscle, adipose tissues. Once insulin arrives at the target cell, it binds with the α

subunit of insulin receptor.(Pederson TM et al 2001, Schwartz MW etal 1992)[126,127] Binding insulin the α subunit stimulates the tyrosine kinase activity of β chains.

The activated tyrosine kinase then autophosphorylates the β chains. The phosphorylated receptor then phosphorylates the tyrosine residues on Insulin Receptor Substrate .(Flakoll PJ et al 2000)[128] Insulin Receptor Substrate phosphorylations are followed by various cascades of events:

1. Gene expression in the nucleus of target cell leading to biological actions
2. Translocation of glucose transporters to the plasma membrane results in insulin mediated glucose uptake.
3. Activation /deactivation of numerous enzymes in glucose and Fatty acid metabolism.
4. Promotes protein synthesis.

2.14.7. ACTIONS OF INSULIN

The insulin have a master role in the metabolism of carbohydrates, lipids, proteins with its target for action as muscle, liver and adipose tissue. Insulin increase potassium, phosphate and magnesium uptake into skeletal muscle and potassium & Phosphate into the hepatic cells from

extra cellular fluid by increasing the membrane permeability. Anabolic action of insulin promotes normal growth and development. Insulin stimulates other growth factors like Insulin like growth factor I & II, Nerve growth factor, EGF and relaxin.(Berne & Lewy) [52]

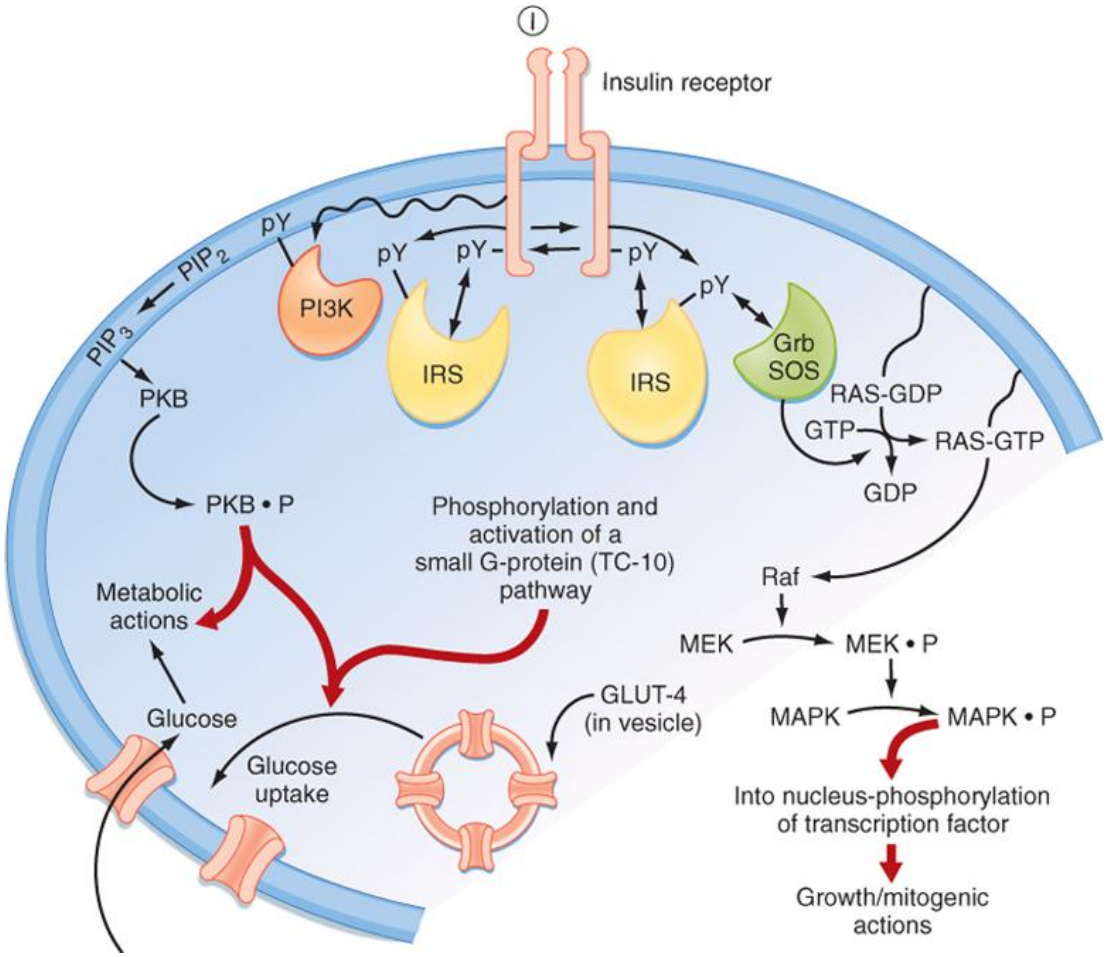


Figure 3 : MECHANISM OF ACTION OF INSULIN

2.14.8. INSULIN AND BRAIN

It is believed that both neuron and pancreatic β islets have evolved from a common ancestral neuron that produced insulin since they share

some similarities. (Rulifson SK, SK Kim et al 2002)[129] Insulin enters the central nervous system through the circumventricular areas without the blood brain barrier and by the action of a specific insulin receptor that transports insulin into brains with the blood brain barrier.(SC Woods, R.J.Seelay et al 2003)[130]

Secretion of Insulin from pancreatic β cells determines its concentration in the periphery. Endothelial facilitated transport across the blood brain barrier into Cerebrospinal fluid influences the brain insulin levels. These are controlled independently of variations of plasma insulin. (Havrankova et al 1979)[136]

Endothelial saturation is the main factor limiting the Insulin translocation during acute hyper insulinemia. Chronic hyperinsulinemia results in receptor internalization. There is evidence from the research studies that high insulin in blood leads to hyper insulinemia in Cerebrospinal fluid.(Wallum BJ et al 1987)[137]

Insulin also plays a role as neuronal survival factor and protects against the toxic effect of AMPA, oxygen and glucose deprivation and to prevent neuronal apoptosis. Therefore, when these protective effects of insulin have been lost, there may be increased risk of neurodegeneration.(M.Schafer, S.L.Erdo et al 1992, J.G.Mielke &

T.W.Yu 2005)[134, 135] The decline of insulin receptor mRNA within the substantia nigra compacta which is the site of neurodegeneration in Parkinson's disease is being demonstrated without involving the insulin levels. (Craft & Watson 2004)[17]

Tau is a microtubule associated protein that is responsible for the stabilization of microtubules inside the axons. Tau influences an effective axoplasmic flow , neuronal connections and signal transmission. Insulin plays a pivotal role in regulating Tau function. Increased levels of insulin load leads to Tau phosphorylation and neuronal degeneration.(S.Freude, L.Plum et al 2005, M.Hong, V.M.Y.Lee et al 1997) [138,139]

2.14.9. INSULIN RESISTANCE

It is the physiological state in which cells fail to respond to the normal actions of insulin. In this condition, body produces insulin, but the cells become resistant to insulin and unable to utilize it. This results in hyperglycemia which leads to production of insulin from β cells further contributing to hyperinsulinemia.

Insulin resistance may be due to mutation in insulin receptor or its kinase activity, down regulation of insulin receptor or alteration in the second messenger transduction.

2.14.10. DIAGNOSIS

1. Fasting serum insulin levels more than upper limit of normal considered evidence for insulin resistance.
2. Glucose tolerance test is normal or mildly impaired in insulin resistance.
3. Hyper insulinemiic euglycemic clamp – this is the gold standard test for measuring and quantifying the insulin resistance.
4. Modified insulin suppression test – this is the alternative test to assess the insulin resistance developed by Corald Reaven at Stanford University.
5. HOMA –IR & QUICKI TEST.

Homeostatic Model Assessment of Insulin resistance and Quantitative Insulin sensitivity Check Index are the simplified tests to measure the Insulin Resistance. Both fasting insulin and glucose levels are used to calculate the insulin resistance. (Matthews DR 1985)[144]

2.14.10. INSULIN RESISTANCE & NEURODEGENERATION

Possible mechanisms explaining the role of insulin resistance in Neurodegeneration are (Arthur F Schuh et al 2011)[145]

1. Sensitization of neurons to toxins and other insults in the presence of high insulin levels

2. Decrease transportation of insulin into brain

3. Tau phosphorylation caused by the brain insulin resistance

4. Brain localized hypoglycemic states due to insulin resistance.

2.14.11. INSULIN RESISTANCE & PARKINSON'S DISEASE

Many clinical studies have demonstrated an association between Insulin Resistance and Parkinson disease since impaired glucose tolerance has been documented in > 50% of parkinsonism patients. (Sandyk 1993) [146]

Diminished insulin mediated uptake of glucose, inhibition of initial secretion of insulin and presence of chronic hyperinsulinemia and hyperglycemia after loading the glucose have been reported in newly diagnosed untreated patients with Parkinsonism. (Von Woert & Muller 1971; Boyd et al 1979)[140,16] Levdopa used in the management of Parkinsonian illness produces both hyperglycemia and hyperinsulinemia.

The association between insulin resistance and Parkinson's disease is best explained by the common pathophysiological pathway (i.e.) the GIGYF2 gene involved in signaling pathway now has been identified as

Park 11 gene. (Eva schern Hammer, Jonni Hansen et al 2011)[142] The relationship between the insulin resistance and Parkinson's disease and the impact of co-morbidity on their pathogenesis is not clear till now.(Morris JK 2010)[39]

Insulin receptors are abundantly present in the dopaminergic nerve cells of substantia nigra Pars compacta (Unger et al 1991) [143] which is evident from the histopathological studies of patients with Parkinsonian illness. Insulin resistance leads to loss of insulin receptor mRNA which coincides with the loss of tyrosine hydrxylase mRNA.(Mooroo et al 1999) [147] Tyrosine hydroxylase is the rate limiting enzyme in dopamine synthesis. Thus, insulin resistance affects the synthesis of dopamine in substantia nigra pars compacta.

In animal models, elevation of glucose concentration in blood leads to suppression of firing of dopaminergic nerve cells in the substantia nigra pars compacta.(Craft & Watson ,2004)[17] It is found that there is a profound reduction of metabolism of dopamine in nigrostriatal tract and olfactory tubercle on administration of glucose to rat.(Montefucso et al 1983)[148]

Chronic hyperglycemia out of insulin resistance results in oxidative stress and produces reactive oxygen species. Insulin resistance leads to

the synthesis of oxygen free radicals which may result in the dopaminergic nerve cell loss. The above discussed two scenarios including the loss of nigrostriatal dopaminergic nerve cells and development of insulin resistance are mediated by oxidative mechanisms. So, insulin resistance and neurodegeneration in Parkinson's disease might be linked through oxidative stress.

Even though glucose uptake in neuronal cell is independent of Insulin, protein involved in insulin signaling such as IRS2 (Insulin Receptor substrate) & GLUT 4 are abundantly concentrated in basal ganglia. So, the altered insulin signaling may impair nigrostriatal dopamine.(Jill & Morris et al 2013) [39] The association between the insulin resistance and Parkinson's disease highlighted the development of insulin sensitizing drugs such as thiozolidinediones and PPAR γ as novel and possible therapeutic agents for Parkinson's disease.

A.E.Boyd, III. Harolde, Lebovitz et al (1979) evaluated disturbances in hormones activity and glucose metabolism in patients with Parkinsonism. They did plasma glucose, insulin and Growth hormone levels in response to oral glucose, IV glucose and IV insulin before and after L.dopa therapy. 44% of control group patient showed an

inhibition of initial secretion of insulin, hyperglycemias, hyper insulinemia after loading the glucose .[16]

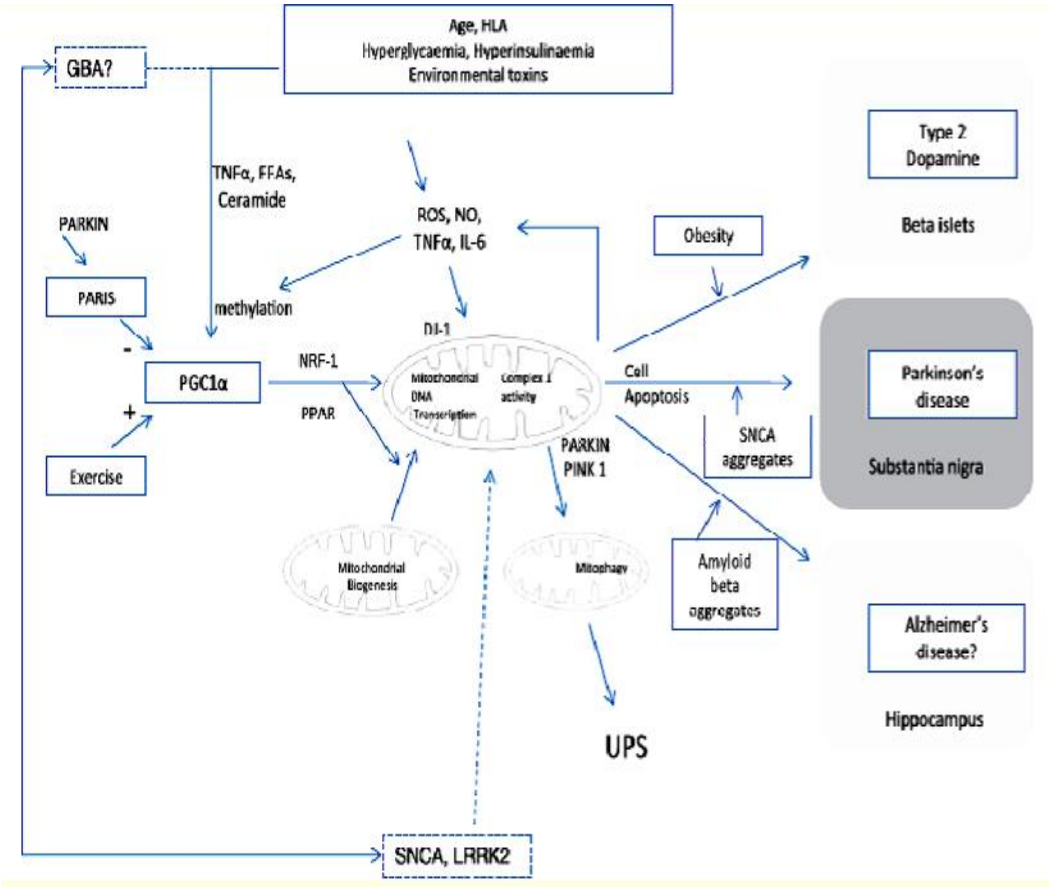


Figure 4 : SCHEMATIC OVERVIEW OF EMERGING PATHWAYS CONNECTING INSULIN RESISTANCE & NEURO DEGENERATION

FFA - Free fatty Acid, HLA - Human leucocyte antigen, IL-6 – Interleukin 6, NO- Nitric oxide, UPS- ubiquitin proteosome system, SNCA- α synuclein, PGCI α - PPAR γ receptor coactivator, LRRK2 – Leucine rich repeat kinase,

Ivan J lipman, Michael E, Boykin et al (1974) studied oral glucose tolerance test in unselected group of Parkinson's patients. The higher values of blood sugar found in Parkinson's patients which was independent of age, duration of illness, severity of disability. Blood sugar values are more when compared to random population of similar

age. 52.4% of group of 63 consecutive Parkinson's patients revealed impaired glucose intolerance.

Domenico Bosco, Massimilliano plastino et al (2012) studied carbohydrate metabolism in 110 Parkinson's disease patients with or without dementia. They evaluated the insulin resistance, glucose, insulin levels after 2 hour oral glucose tolerance test in 53 Parkinson's disease patients with dementia and 57 Parkinson's disease patients without dementia. The insulin resistance was highly prevalent in 62% of Parkinsonism patients with dementia of which 30% had impaired glucose tolerance, 5.6% were newly diagnosed diabetes mellitus and 26% had only insulin resistance. The prevalence of insulin resistance in Parkinson's patients with dementia was found to be twice than that of Parkinson's patients without dementia.[74]

Morris JK, Bornhoff GL et al (2010) studied the relationship between the neurodegeneration and insulin resistance in a preclinical animal model of Parkinson's disease. High fat feeding is an established animal model of insulin resistance. Higher HOMA IR indices & diminished insulin mediated glucose uptake support the abnormal insulin sensitivity. Animals fed in the high fat diet group showed profound Dopamine secreting nerve loss in the Nigrostriatal pathway. Peripheral

insulin resistance would exaggerate the toxin induced nigrostriatal dopaminergic neurodegeneration.[39]

M.H.Van woert, P.S.Muller et al (1973) studied on insulin secretion and glucose metabolism in Parkinson's disease patients before and during treatment with L-dopa compared with age matched controls. The Parkinson's disease patients showed that disappearance rate of glucose was very less and a response of insulin to IV glucose was also very less which were not modified by treatment with L-dopa. Their findings support a defective mechanism in the IV glucose mediated insulin release in Parkinson's disease patients.[140]

Borrislav Ivanov, Ara Kaprelyan et al (2012) studied the link between the pathophysiological pathways of parkinsonian illness and Type 2 diabetes mellitus in 85 Parkinson disease patients. They found the prevalence of insulin resistance in Parkinsonism is 18.8%. They suggested that changes in glucose control was associated with loss of dopaminergic neurons which may occur in the early phase of disease.[121]

Gang Hu,Peek Jous J lahti et al (2007) prospectively studied the 51,552 men and women in 25-74 years of age without history of Parkinson's disease at baseline. During the course of 18 years, 324 men and 309 women with impaired glucose tolerance developed incident Parkinson's disease.

F Federico, IL Simone et al (1997) performed Proton Magnetic resonance spectroscopy in 8 patients with Parkinson's disease which revealed abnormal glucose utilization and striatal neuronal loss in Parkinson disease.[141]

Sandyk (1993) studied the impact of chronic hyperglycemia, abnormal glucose intolerance on the severity of disease and the course of illness as it has been documented that 50 % to 80% of Patients with Parkinson's disease revealed impaired glucose tolerance. Insulin resistance might exacerbate the severity of illness in Parkinson's disease and levodopa induced dyskinesias. He suggested that Parkinson's disease patients should be routinely examined for the evidence of glucose intolerance and that if found aggressive management of the insulin resistance and hyperglycemia diminish the severity of the disease.[146]

From the above studies it is inferred that the prevalence of Insulin resistance is higher in patients with Parkinson's disease due to many reasons. When it is associated with Parkinson's disease it may increase the severity of the illness due to accentuated neurodegeneration and it throws light into the development of novel threpeutic agents for Parkinson's disease.

3. AIM & OBJECTIVES

AIM

To evaluate the Resting Heart Rate Variability and serum Insulin levels in patients with Parkinson's disease.

OBJECTIVES:

1. To evaluate the resting cardiac autonomic activity in patients with Parkinson's disease using Heart Rate Variability at rest and to be compared with the healthy subjects.

2. To study the prevalence of Insulin resistance in Parkinson's disease patients by evaluating serum insulin levels.

3. To correlate Insulin resistance with Heart Rate Variability variables in Parkinson's disease patients.

4. MATERIALS AND METHODS

The study was conducted in the Institute of Physiology and Experimental Medicine, Madras Medical College and Rajiv Gandhi Government General Hospital, Chennai-3, after getting permission from the Institutional Ethical Committee, Madras Medical College, Chennai – 3.

4.1. PATIENT SELECTION

Patients of both sexes in the age group between 40 – 70 years fulfilling the criteria for Parkinson's disease as per the Parkinson's disease Society Brain Bank Clinical Criteria were included in the study. Hoehn and Yahr scale was used to assess the severity of motor impairment in this study.

They were selected from the Institute of Neurology, Rajiv Gandhi General Hospital, Chennai - 3. All the Participants included were informed about the study and a written and informed consent were obtained from them.

Detailed history including symptoms of autonomic dysfunction such as giddiness on standing / near syncope, palpitation, symptoms of bladder disturbances such as retention, hesitancy, urgency, incontinence

of urine, features of bowel disturbances such as constipation, diarrhoea, loss of libido were elicited. All the participants were subjected to complete general and systemic examination.

4.2. INCLUSION CRITERIA

30 Patients with Parkinson's disease of the age group between 40 – 70 years were included in the study.

4.3. EXCLUSION CRITERIA

- Patients with atypical Parkinsonism, Parkinson plus syndrome, Multisystem atrophy, Supra nuclear palsy
- Patients with Secondary Parkinsonism (vascular, traumatic, drug induced)
- Patient with diabetic autonomic neuropathy
- Patent taking medication that may act directly on ANS (calcium channel blocker, sedative etc)
- Patient with cardiovascular diseases
- Patient with impaired hepatic, renal function
- Patient with severe mental illness
- Patient with concomitant neurological & musculoskeletal disorder

4.4. SELECTION OF CONTROLS

The Control group consisted of 30 healthy subjects (men, women) with the age between 40-70 years were included in the study after history taking, clinical examination and laboratory tests.

4.5. LABORATORY ASSESSMENT

Laboratory investigations include Haemoglobin, Fasting blood sugar, postprandial blood sugar, blood urea, serum creatinine. Insulin resistance is studied by Fasting serum insulin and Fasting blood sugar.

4.6. MATERIALS

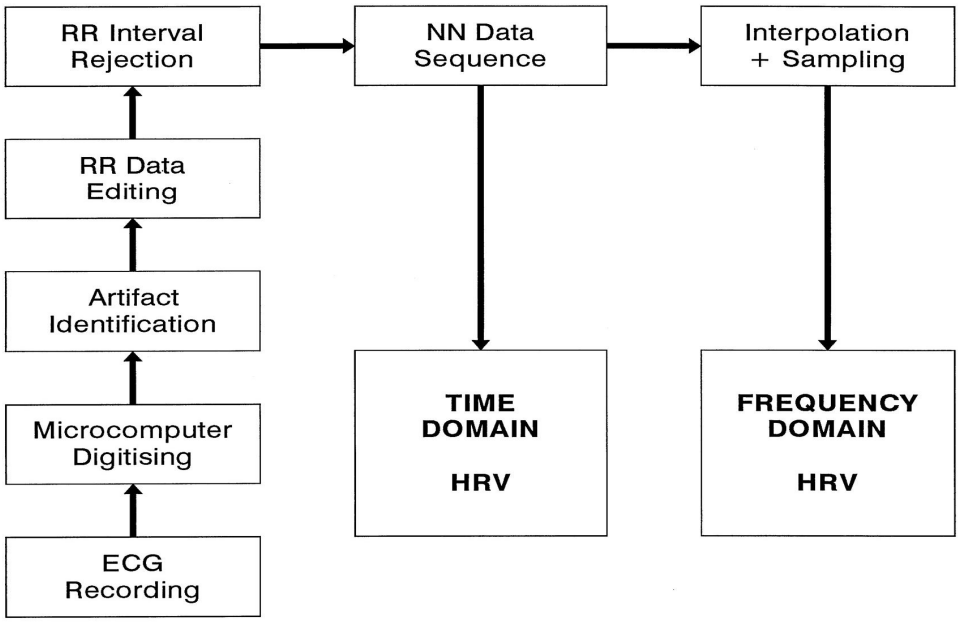
- Niviqure ambulatory digital ECG recorder (INCO)
- Pulse rate and NIBP recorder – PLANET -50
- Sphygmomanometer

4.7. EQUIPMENT

Niviqure is a solid state, multi load, digital, stand alone computerized recording system designed to acquire, analyze and store ECG data over long hours. This data is acquired and stored in flash memory for later downloading and analysis. The data transfer from

memory module and computer is through an interface RS233C, compatible module.

Niviqure has powerful processing software for online ECG study, data storage, off line data replay and study and data transfer to other software for statistical analysis and FFT analysis. Flow chart given below summarizes the individual steps for obtaining the information for HRV analysis.



4.8. MEASUREMENT OF HEART RATE VARIABIITY

HRV analysis gives a non-invasive estimate of the function of sympathetic and Parasympathetic nervous system of heart. Either the heart rate as a function of time or the period of time between successive QRS complexes needs to be quantified in HRV analysis.

4.9. HRV ASSESSMENT

Two types of recording are commonly used for the standardisation of electrophysiological and clinical studies, two types of recording are commonly used (Task Force)[66]

a. Short term recording (5 min) – computed by frequency domain methods.

b. Long term recording (24 hour) - computed by time domain methods.

HRV can be assessed either for a short term (5 minutes) or for a long term (24 hours). Frequency domain methods are more preferable for short term recording and time domain methods are preferred for long term recording. (Task Force,1996)[66]

A good quality of ECG recordings without artefact RR interval tachogram consisting of only normal to normal of RR interval resulting from sinus node depolarization is taken for HRV analysis. HRV is analyzed using the Finland software IV of version 1.1, Microsoft window based PC that gives the report sheet with time domain and frequency domain variables for various events which are already marked in the ECG.

4.9.1. TIME DOMAIN METHODS

The simplest that can be calculated directly from the raw RR interval time series are the time domain methods. The mean HR and mean RR and standard deviation of the RR intervals are the simplest time domain measures.

The simplest time domain measures are

Mean HR – mean of the selected RR interval

Mean RR- mean of the selected RR series.

The overall variation in the RR interval signal is best described by SDNN that is standard deviation of NN intervals. The short term variation are best described by standard deviation of consecutive differences between consecutive RR intervals (SDSD). NN50 is the other commonly used parameter which is calculated from the number of consecutive RR intervals differing more than 50 ms.

The percentage value of NN50 intervals is pNN50. The normal-to-normal intervals (i.e. intervals between consecutive QRS complexes resulting from sinus node depolarization) is given by the prefix NN. In

practice, RR and NN intervals usually share the same meaning. (Task Force,1996)[66]

4.9.2. STATISTICAL METHODS

The more complex statistical time domain measures could be calculated from a series of instantaneous heart rate or cycle length intervals which are recorded over longer period, usually for 24 hours,. These can be divided into (i) those calculated from direct measurement of NN intervals or instantaneous heart rate and (ii) those computed from the difference between NN intervals.

The SDNN is the standard deviation of these Normal to Normal intervals which represents the total power of spectral analysis. This is the simplest variable to be calculated using statistical methods. Unit of SDNN is given in milliseconds. SDNN act as a universal index of HRV. All the long term components and circadian rhythm which is causative for the variability in the recording period is best represented by SDNN. (Saul JP, Albrecht P et al 1993)[97]

The standard deviation of the averages of NN intervals in all 5 minute segment of the full recording gives SDANN. The variations of the average of NN intervals in 5 minute intervals over 24 hours is given

by SDANN. Thus, it gives information on long term components and it is a more valuable indicator of low frequencies like physical activity, changes in state of body and circadian rhythm. Another most reliable index is pNN50 that measures what percent of the inter beat intervals differ from the neighboring intervals by 50 milliseconds or more and it is expressed in percentage.(Task Force,1996)[66]

SD or SDSD represents the standard deviation of differences between adjacent NN intervals. RMSSD is another variable calculated from the square root of the mean of the sum of the squares of difference between adjacent NN intervals. It corresponds to high frequency variation in short term NN recordings which gives an estimate of vagal modulation of the heart. Unit of RMSSD is milliseconds. (Task Force,1996)[66]

4.9.3. FREQUENCY DOMAIN VARIABLES

The fundamental information regarding the distribution of the power (variance) as a function of frequency is best given by Power spectral density (PSD) analyzer in frequency domain, in spite of various spectral methods for studying R-R tachogram. With the use of proper mathematical algorithms, an estimate of true power spectral density can be obtained. They can be divided into

Non parametric methods (Fast Fourier Transform)

Parametric methods (Auto regressive Models, Marple1987) [93]

The method of decomposition of periodic oscillations of heart rate signal at different frequencies and amplitude is best given by frequency domain analysis and gives valuable information on their relative intensity (termed variance or power) in the sinus rhythm of heart. Frequency domain methods are more preferred when compared to the time domain methods especially with the analysis of short term recordings. (Task Force, 1996)[66]

The Power Spectral Density (PSD) analysis is done by computing the powers and frequencies for various frequency bands. The commonly used frequency bands are as follows: very low frequency (VLF, 0-0.04 Hz, low frequency (LF, 0.04-0.15 Hz), and high frequency (HF, 0.15-o.4 Hz).(Sayers BM 1973; Hirsh JA, Bishop B et al 1981) [88]

Parameter	Definition	Frequency Range
Total power	Variance of NN intervals	<0.4 Hz
ULF	Ultra low frequency	<0.003 Hz
VLF	Very low frequency	0.0 – 0.04 Hz
LF	Low frequency	0.04 – 0.15 Hz
HF	High frequency	0.15 – 0.4 Hz

ULF: Includes the circadian rhythm.

VLF: Supposed to be influenced by temperature regulation and humoral systems.

LF: Affected by changes in cardiac sympathetic (and presumably Parasympathetic) activity.

HF: Sensitive to changes in respiratory rhythm and primarily influenced by cardiac parasympathetic activity.

The usual parameters to be analyzed in frequency domain are found to be the powers of VLF, LF, and HF bands in absolute and relative values, and the normalized power of LF and HF bands, and the LF / HF ratio. For each frequency band, the peak frequencies are calculated too. By integrating the spectrum over the frequency bands, spectrum powers are given for the Fast Fourier Transformer (FFT).(Task Force,1996) [66]

A continuous smooth spectrum of activity is observed in the parametric method (Autoregressive model) results which is more complex and has to be verified with the suitability of chosen model.(Task Force,1996) [66]

4.10. SPECTRAL COMPONENTS

SHORT TERM RECORDING

In this study we have performed only short time HRV recording that for 5 minutes. In non parametric method, Fast Fourier Transform (FFT) is used as algorithm. This is very simple and rapid. It has a high processing speed. The Power Spectral Component has recordings ranging from 4-400 MHz. (Sayers BM 1973; Askerold et al 1981; Pagani M,Lombardi F et al 1986)[85,88,99]

The three main components that are distinguished in a spectral calculation for short term ECG recording are

VERY LOW FREQUENCY (VLF)

LOW FREQUENCY (LF)

HIGH FREQUENCY (HF)

There is no constant distribution of the power and central frequency of LF and HF but they may vary in relation to changes in the sympathetic and parasympathetic modulation of the heart. The most commonly accepted component of VLF is believed to be the non

harmonic component with no coherent properties which is influenced by algorthim of baseline or trend removal.

LF and HF can be calculated in normalized unit. The measurement of LF and HF in normalized unit best explains the coordinated and balanced behavior of Autonomic Nervous system. Normalized unit means the relative value of each LF, HF power component in proportion to the total power minus the VLF component (TP-VLF).(Task Force1996)[66]

HF component is believed to be a quantitative a marker of parasympathetic (vagal) modulation. This HF component is mainly mediated by respiratory movements and it is influenced by the frequency of breathing. The LF component is influenced by both sympathetic and vagal modulation. LF/HF ratio is a strong indicator of sympatho-vagal balance.

4.10.1 . VERY LOW FREQUENCY VLF (0-0.04Hz)

There is much less defined physiological basis for the VLF component and the existence of a specific physiological process influencing these VLF component might even be questioned. Thus VLF computed from short-term HRV analysis(e.g. 5 min) is a dubious

measure and should be avoided while analyzing the PSD of short-term ECGs. Therefore, in the present study only LF and HF was studied. (Malliani et al 1991)[96]

4.10.2. LOW FREQUENCY LF (0.04-0.15Hz)s

This is an important indicator for more sympathetic than parasympathetic modulation. This requires a minimum of 2 minutes recording.

4.10.3. HIGH FREQUENCY HF (0.15-0.4Hz)

This power spectral oscillation is mainly seen only for parasympathetic nervous system. Hence, this is specially blocked by parasympatholytic drugs. It can be calculated even with 1 minute ECG recording.

4.10.4. LOW FREQUENCY/HIGH FREQUENCY RATIO: (LF/HF RATIO)

LF/HF ratio has been used as a better non – invasive index of global sympathovagal balance. Interpretations of these HRV indices mainly rely upon the existing physiological state. (Pagani M et al 1986) [95]

4.10.5. NORMALIZATION OF UNITS

LF (n.u) and HF (n.u) are the normalization of the powers that gives near 100% values of the sympathetic and parasympathetic system. They can be calculated as follows (Task force, 1996)

LF (n.u) = LF power / (LF + HF power) or LF power/ (TP –VLF)

HF (n.u) =HF power/ (LF+HF power) or HF power/ (TP-VLF)

Total power = LF power + HF power

4.11. METHODOLOGY

The sixty age and sex matched study and control group were subjected to assess resting heart rate variability using Niviqure ambulatory digital ECG recorder.

Many endogenous and environmental factors can confound the autonomic testing and need to be controlled. The following precautions were taken while recording the Heart rate Variability.

- The subjects should be calm, comfortable, mentally relaxed and free from recent acute illness, and without significant anxiety.

- The Heart rate variability was recorded between 10 -12 AM, 2 – 3hours hours after breakfast.

- Subjects were asked to remove any Compressive garments.

- Caffeine, nicotine and alcohol were avoided on the day of recording Heart rate variability.

- Vigorous exercise was avoided on the day prior to the test.

- Any other medication that could modulate the blood pressure was discontinued ideally 24-48 hours prior to recording.

- The subjects were asked to empty the bladder before the test.

- The test was performed in quiet room with controlled temperature ranging from 25-28 degree Celsius lighting subdued.

- All mobile phones, electronic items weres kept 1 metre away from the recording site

Photograph 1- Niviqure ambulatory digital ECG recorder

4.12. RESTING HRV RECORDING

- After explaining briefly about the study, the informed consent from the subjects are obtained.
- Height in meters and weight in kilograms were measured and their BMI were calculated.
- Patients were instructed to lie down in supine posture and relax for a period of 10- 15 minutes.
- Resting heart rate and blood pressure were recorded.
- Electrodes were placed in the position as given below after cleaning the site with spirit.

PLACEMENT OF ELECTRODES

ELECTRODE	POSITION
Exploring electrode	Lt Shoulder
Exploring electrode	Rt Shoulder
Exploring electrode	Lt subcostal
Reference electrode	Rt Subcostal

- Resting HRV was recorded in the supine position using ECG recorder.

- It was recorded for 320 seconds which is needed for short term HRV analysis.

Photograph 2- Resting HRV analysis recording

- After screening for the artifacts and editing it, the results obtained were fed to the HRV analysis software.

- The analogue to the digital conversion of the resting ECG signals was done by a AD converter with a sampling frequency of 1024/ sec.

- The Power spectral analysis of the converted ECG signals was done by using Fast Fourier Transformation

4.13. PARAMETERS STUDIED

Mean RR, Mean HR, SDNN, RMSSD, NN50, pNN50, LF power, HF power, LF/HF ratio, Total Power were estimated. The values are Compared with the normal values obtained from the control group. The results of these tests are evaluated statistically.

4.14. ASSESSMENT OF SERUM INSULIN LEVELS

- Under aseptic precautions about 5ml of blood was collected from the patient and controls after 10 hours of fasting.

- The blood was centrifuged to separate the serum.

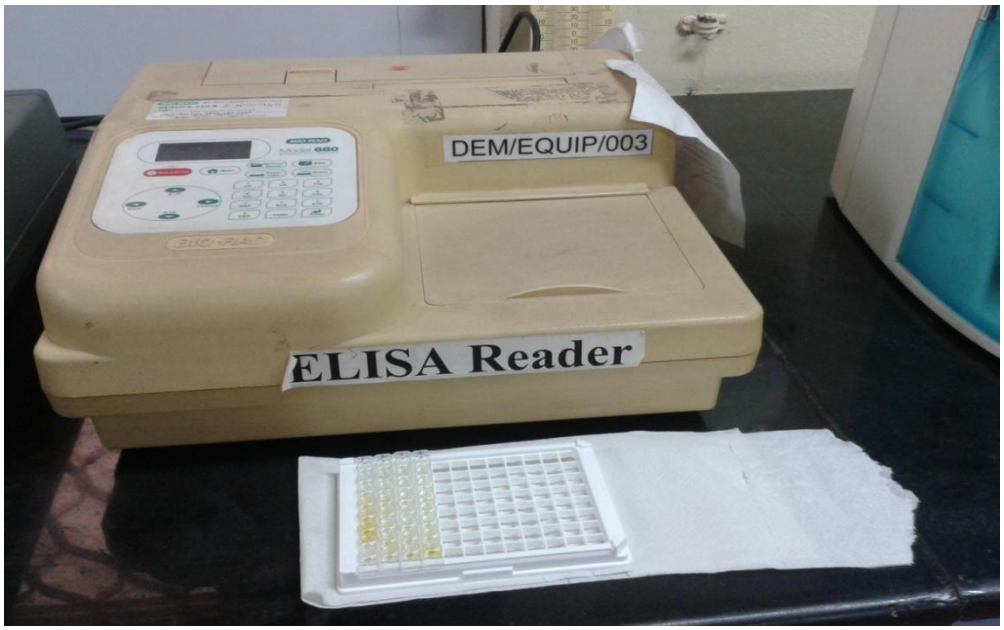
- The serum samples were stored at -20 degree Celsius.

- **ACCUBIND Elisa microwells Insulin Test system** was used for assessing the serum Insulin levels.

4.15. MATERIALS REQUIRED

1. Insulin calibrators – six vials of references for Insulin antigen at levels of 0,5,25,50,100,300 μ IU / ml

Photograph 3- Insulin kit



Photograph 4 –ELISA reader at TNMGR University

2. Insulin enzyme reagent – containing enzyme labelled affinity purified monoclonal mouse x-insulin IgG, biotinylated monoclonal mouse insulin IgG in buffer

3. Streptavidin coated plate – 96 wells

4. Wash solution concentrate

5. Substrate A & Substrate B

6. Stop solution

4.16. INSULIN ASSAY PROCEDURE

- Wash buffer solution and working substrate solution were prepared.
- 50 µl of the appropriate calibrators, controls and samples were pipetted into the assigned wells.
- 100 µl of the Insulin Enzyme Reagent was added to each well.
- The microplate was gently swirled for 20 -30 seconds for proper mixing.
- The microplate was incubated for 120 minutes at room temperature.

- The contents of microplate were discarded either by decantation or aspiration.
- 350 µl of wash buffer was added and then decantated or aspirated.
- Three washes were repeated using automatic or manual plate washer.
- 100 µl of working substrate solution was added to all wells and mixed gently for 15-20 seconds.
- The absorbance was read in each well at 450 nm in a microplate reader.

4.17. CALCULATION OF RESULTS

A dose response curve was used to determine the concentration of Insulin.

1.The absorbance for each duplicate serum reference and the corresponding Insulin concentration in µl/ml were plotted on a graph paper.

2. The best fit curve was selected from the plotted points.

3. The average absorbance of the duplicates for each unknown solution was plotted on Y axis, the corresponding insulin concentration is obtained from the X axis. The normal value of serum insulin in adult is 0.7 – 9 μIU /ml.

4.18. HOMA-IR (HOMEOSTATIC MODEL ASSESSMENT-INSULIN RESISTANCE)

The Homeostatic Model Assessment is a new method used to quantify insulin resistance which was first described by Matthews et al in 1985. It was derived from the data of the various physiological studies using the mathematical equation to describe the glucose regulation.

$$\text{HOMA-IR} = \frac{\text{Glucose X Insulin}}{405}$$

(Glucose in mass units mg/dl), IR – Insulin resistance, Blood for insulin and glucose are taken during fasting. HOMA-IR > 2 – 2.5 considered as significant for insulin resistance in non-diabetic population.

5. RESULTS

The statistical analysis of the data obtained from conducting the Heart Rate Variability test and serum insulin levels were done using the statistical Package for the social sciences (SPSS) software version 17. The mean and standard deviation of the variables were determined for both the groups. Independent student t test was employed as the test of significance at 95% confidence interval. P value <0.05 was considered as significant.

5.1. CHARACTERISTICS OF CONTROL & STUDY POPULATION

The characteristics of control and study population are furnished in the table-1. Table(1)a comprises the age distribution between the control and cases.

TABLE I (a) – AGE

VARIABLE	GROUP	MEAN±SD	T TEST	P VALUE
AGE	Control	55.9 ± 6.18	0.7236	0.472
	Cases	57 ± 5.58		

P < 0.05 Significant

Among the individuals screened, the mean age of the subjects was found to be 55.9 ± 6.18 years with the age range of 40 to 70 years in the control; whereas the mean age of the Parkinson's disease patients included in the study was 57 ± 5.58 years. The difference in the age among the group was not significant.

Table I (b) comprises the gender distribution between the cases and controls.

Table 1(b) - GENDER

GROUP		MALE	FEMALE	TOTAL
Controls	n	18	12	30
	%	60	40	100%
Cases	n	20	10	30
	%	66.7	33.3	100%
Total	n	38	22	60
	%	63.3	36.7	100%

Among the 30 Parkinson disease Patients, 20 (66.7%) were males and the remaining 10 (33.3%) were females. Similarly, of the 30 control subjects, 18 (60%)were males and 12 (40%) were females. Hence, in this study, distribution of individuals both in the control as well as in the Parkinson's disease group was almost equal and uniform according to gender.

Table 1(c) shows the comparison of BMI between the cases and controls

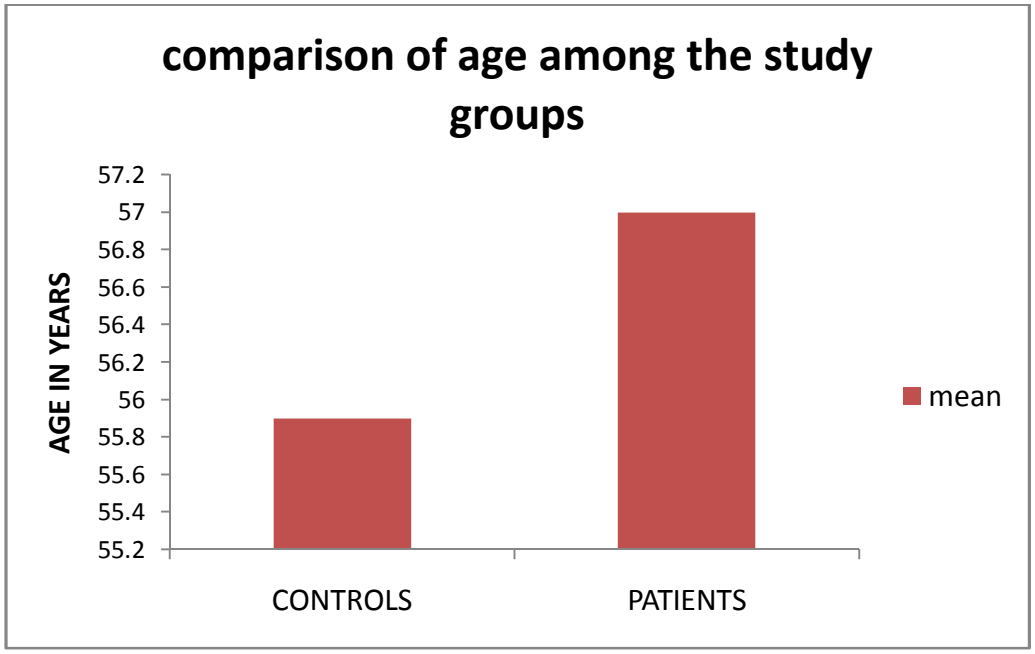
TABLE 1(C) – BMI

VARIABLE	GROUP	N	MEAN±SD	T TEST	P VALUE
BMI	Controls	30	22.08 ± 1.68	1.777	0.08
	Cases	**30**	**21.3 ± 1.71**		

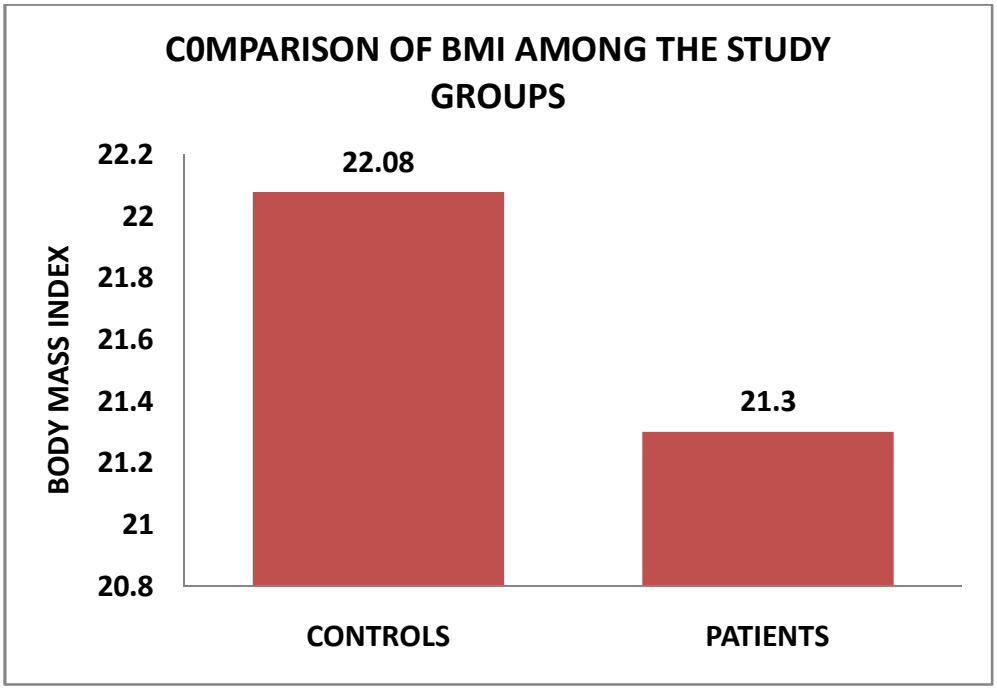
P < 0.05 Significant

The body mass indices were estimated as 21.3 ± 1.71 and 22.08 ± 1.68 for control and parkinson's disease patients respectively and the difference was not statistically significant.

Graph 1 COMPARISON OF AGE AMONG THE STUDY GROUPS



Graph 2 COMPARISON OF BMI AMONG THE STUDY GROUPS



The duration of illness of the Parkinson's disease patients selected were from as low as 1 year to as high as 16 year which averaged 6 ± 4.6 years.

Table 2 (a),(b) compares the resting Heart rate, systolic blood pressure & diastolic blood pressure of the cases and controls.

TABLE 2(a) – RESTING HR

VARIABLE	GROUP	N	MEAN ± SD	T TEST	P VALUE
Resting HR	Controls	30	78.03 ± 5.91	1.858	0.06
	Cases	30	74.9 ± 7.10		

P value < 0.05 significant

TABLE 2 (b) – RESTING SBP, DBP

VARIABLE	GROUP	N	MEAN ± SD	T TEST	P VALUE
SBP	Controls	30	120.86 ± 4.5	3.306	0.001*
	Cases	30	113.06 ± 12.11		
DBP	Controls	30	76.4 ± 4.99	1.863	0.067
	Cases	30	73.53 ± 6.80		

*P value < 0.05 significant

The mean value of the resting HR and the mean value of the DBP between the cases and controls did not differ significantly. However, there was highly significant fall in the mean SBP in the Parkinson's disease patients group.

5.2. RESTING HRV

Both the groups were subjected to the analysis of HRV by continuously recording the ECG in a relaxed supine position for 5 minutes. Variables pertaining to Heart Rate Variability at rest in time and frequency domain between the normal subjects and Parkinson's disease patients are furnished in the table 3 to table 9.

Table 3- comparison of Mean HR & mean RR among the study groups

TABLE -3 MEAN HR, MEAN RR

VARIABLE	GROUP	N	MEAN ± SD	T TEST	P VALUE
Mean HR	Controls	30	74.43 ± 5.04	1.358	0.1794
	Cases	30	72.33 ± 10.25		
Mean RR	Controls	30	0.809 ± 0.06	1.967	0.054
	Cases	30	0.853 ± 0.11		

P value < 0.05 significant

The mean HR of the control was 74.43 ± 5.04 and the Parkinson's disease group was 72.33 ± 10.25. The mean RR of the control group was 0.809 ± 0.06 and that of Parkinson's disease group was 0.853 ± 0.11. Both the values are not statistically significant.

TABLE 4 – COMPARISON OF SDNN AMONG THE STUDY GROUPS

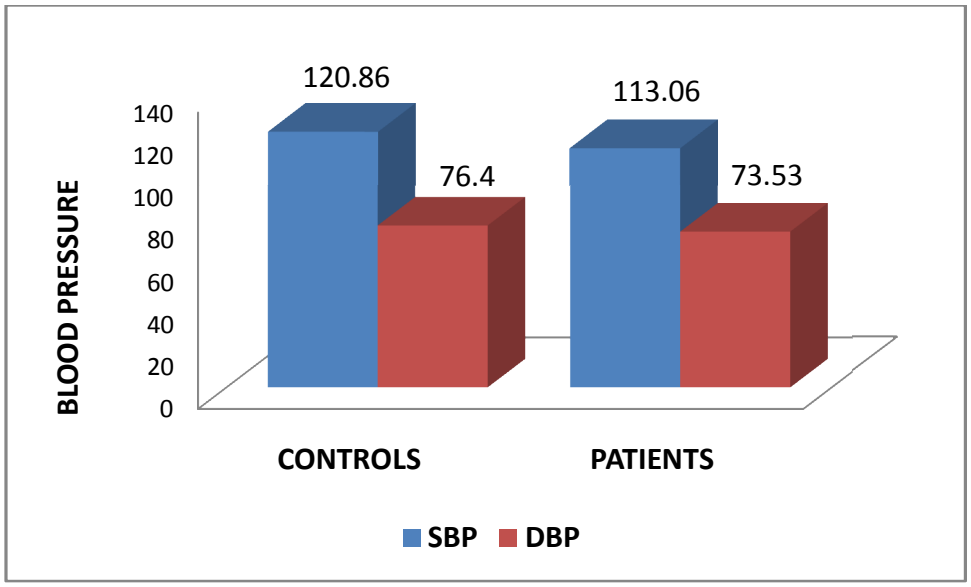
TABLE 4 - SDNN

VARIABLE	GROUP	N	MEAN ± SD	T TEST	P VALUE
SDNN	Controls	30	70.75 ± 38.59	2.198	0.03*
	Cases	30	52.06 ± 26.16		

P value < 0.05 significant

The mean value of the SDNN in the control group was 70.75 ± 38.59 which differ significantly with the mean value of 52.06 ± 26.16 of the Parkinson's disease patients.

Graph 3 COMPARISON OF RESTING BLOOD PRESSURE AMONG THE STUDY GROUPS



Graph 4 COMPARISON OF SDNN & RMSSD AMONG THE STUDY GROUPS

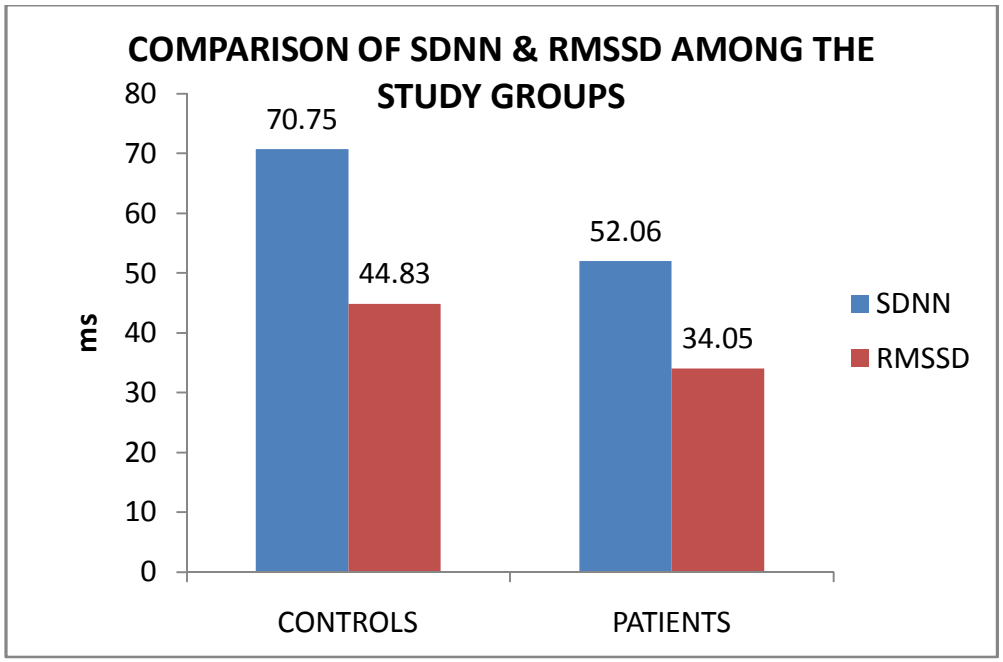


TABLE – 5 COMPARISON OF pNN50 & RMSSD AMONG THE STUDY GROUPS

TABLE-5 pNN50, RMSSD

VARIABLE	GROUP	N	MEAN ± SD	T TEST	P VALUE
pNN50	Controls	30	23.56 ± 12.62	1.358	0.179
	Cases	30	18.12 ± 17.93		
RMSSD	Controls	30	44.83 ± 22.89	2.167	0.03*
	Cases	30	34.05 ± 14.77		

*P value < 0.05 significant

The mean value of the pNN50 in the control group was 23.56 ± 12.62 and the mean value of the pNN50 in the Parkinson's disease group was 18.12± 17.93 which was not statistically significant.

TABLE -6 COMPARISON OF LF (ms^2) AMONG THE STUDY GROUPS

TABLE- 6 LF (ms^2)

VARIABLE	GROUP	N	MEAN±SD	T TEST	P VALUE
LF (ms^2)	Controls	30	171.03± 97.60	3.382	0.001**
	Cases	30	101.7 ± 58.44		

** P value < 0.001 highly significant

The LF power values which is an indicator of sympathetic tone was lower in Parkinson's disease group and was highly significant.

TABLE -7 COMPARISON OF HF (ms^2) AMONG THE STUDY GROUPS

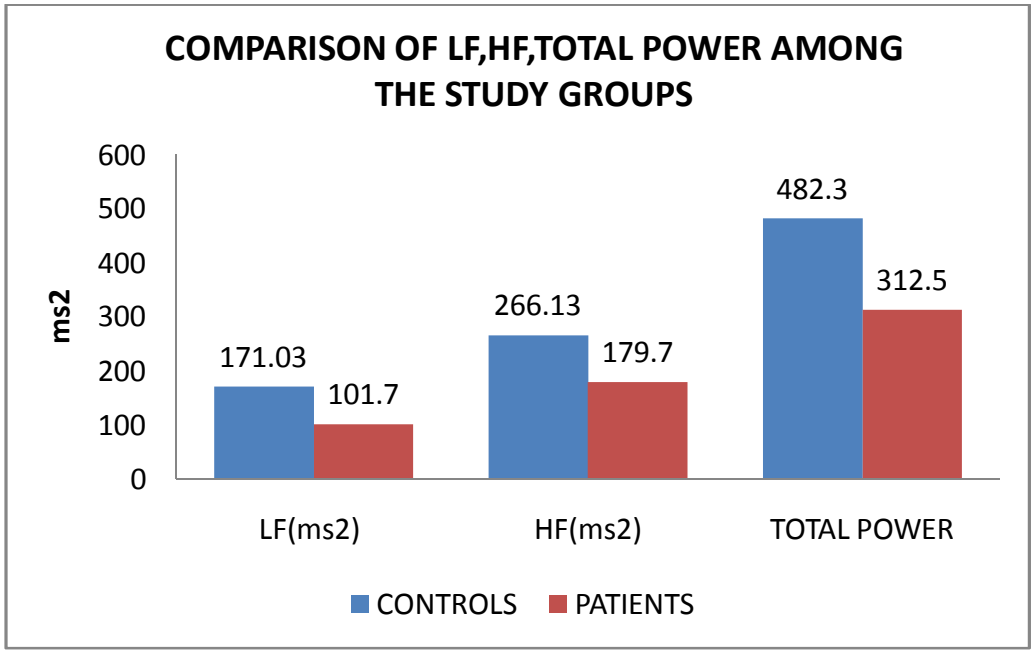
TABLE- 7 HF (ms^2)

VARIABLE	GROUP	N	MEAN±SD	T TEST	P VALUE
HF (ms^2)	Controls	30	266.13 ± 170.74	2.424	0.01*
	Cases	30	179.7 ± 94.7		

* P value < 0.05 significant

The HF power values which is an indicator of vagal tone was lower in Parkinson's disease group and was highly significant.

Graph 5 COMPARISON OF LF(ms^2) , HF(ms^2) & TOTAL POWER



Graph 6 COMPARISON OF LF/HF RATIO AMONG THE STUDY GROUPS

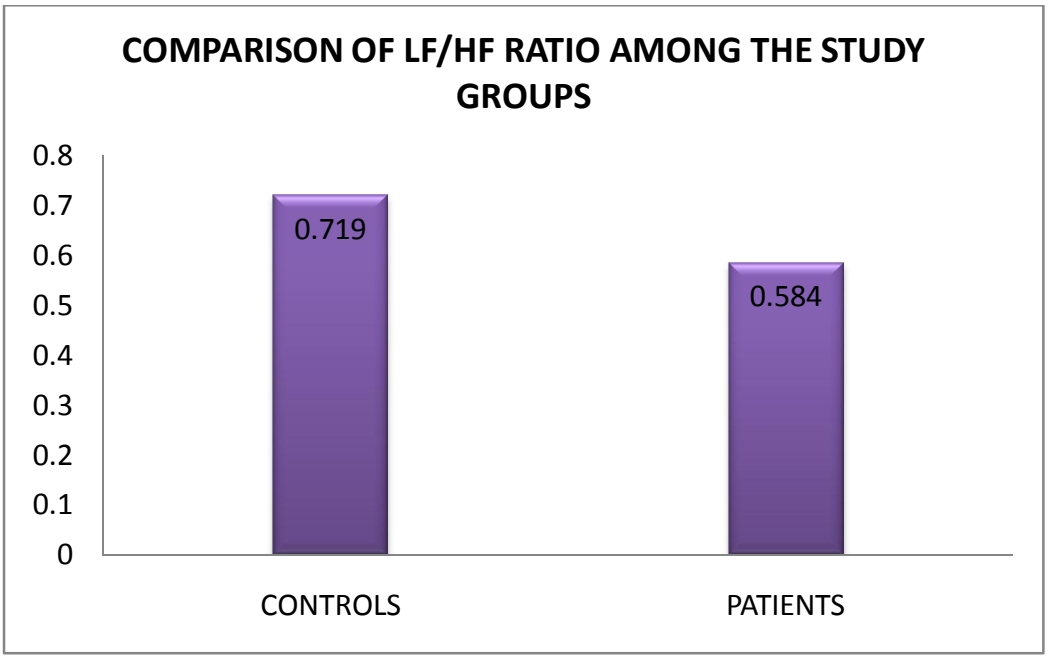


TABLE -8 COMPARISON OF LF/HF RATIO AMONG THE STUDY GROUPS

TABLE - 8 LF / HF RATIO

VARIABLE	GROUP	N	MEAN±SD	T TEST	P VALUE
LF/HFratio	Controls	30	0.719 ± 0.26	2.451	0.01
	Cases	30	0.584 ± 0.153		

* P value < 0.05 significant

The LF/HF ratio which is a global index of sympathovagal balance was significantly lower in Parkinson's disease group.

TABLE -9 COMPARISON OF TOTAL POWER AMONG THE STUDY GROUPS

TABLE- 9 TOTAL POWER

VARIABLE	GROUP	N	MEAN±SD	T TEST	P VALUE
TOTAL POWER	Controls	30	482.3 ± 267.5	3.009	0.003*
	Cases	30	312.5 ± 154.8		

* P value < 0.05 significant

The Significantly reduced total power values indicate the reduced HRV in the Parkinson's disease group.

TABLE – 10 COMPARISONS OF FBS & FASTING SERUM INSULIN LEVELS AMONG THE STUDY GROUPS

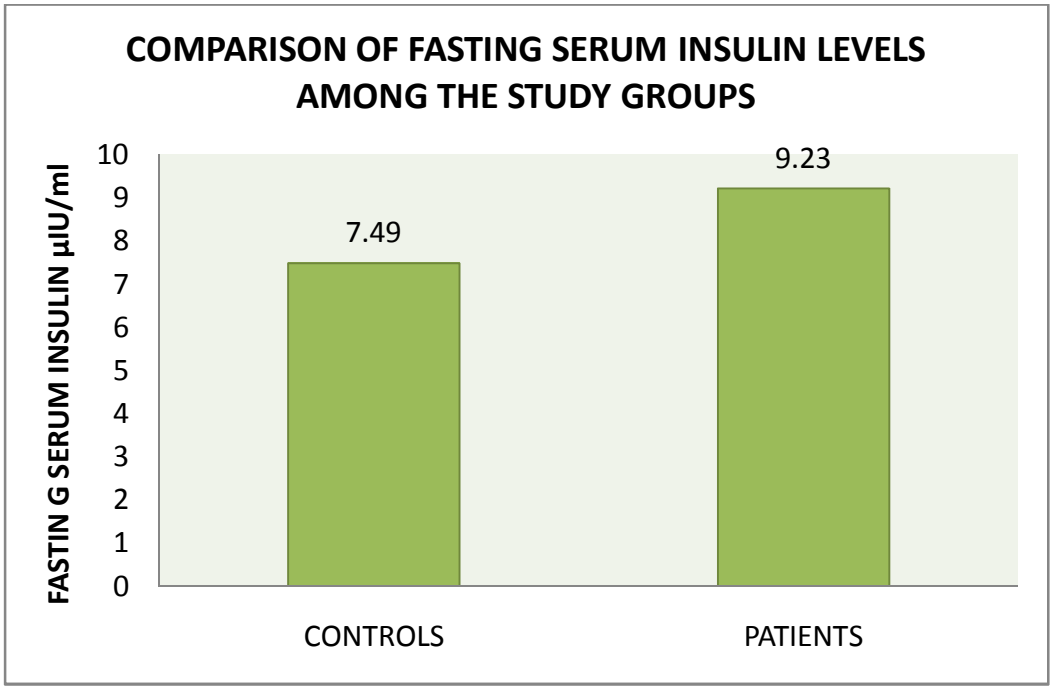
TABLE-10 FBS, FASTING SERUM INSULIN

VARIABLE	GROUP	N	MEAN ± SD	T TEST	P VALUE
FBS	Controls	30	97.3 ± 16.02	1.732	0.08
	Cases	30	103.5 ± 11.3		
SERUM INSULIN	Controls	30	7.49 ± 3.22	2.010	0.04*
	Cases	30	9.22 ± 3.48		

*P value < 0.05 significant

There was no significant difference in the fasting blood sugar among both the study groups. Serum insulin levels were significantly more in Parkinsonian illness patients than the control groups.

Graph 7 COMPARISON OF FASTING SERUM INSULIN LEVELS AMONG THE STUDY GROUPS



Graph 8 COMPARISON OF INSULIN RESISTANCE INDEX VALUES AMONG THE STUDY GROUPS

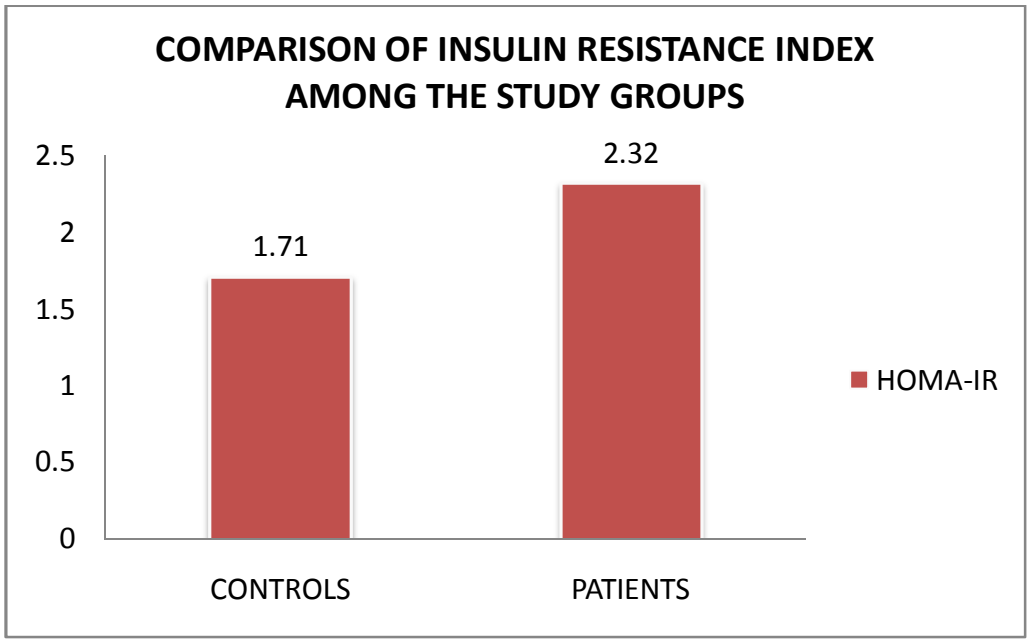


TABLE-11 COMPARISON OF HOMA- IR VALUES AMONG THE STUDY GROUPS

TABLE-11 HOMA – IR (INSULIN RESISTANCE INDEX)

VARIABLE	GROUP	N	MEAN±SD	T TEST	P VALUE
HOMA - IR	Controls	30	1.71 ± 0.61	3.569	0.0007**
	Cases	30	2.32 ± 0.71		

*P value < 0.05 significant

There was a highly significant difference in the P value < 0.001 in the HOMA-IR values between the study groups. HOMA-IR values are significantly high in Parkinson's disease patients.

5.3. CORRELATION BETWEEN HRV PARAMETERS, HOMA-IR VALUES ,FASTING INSULIN AND DURATION OF PARKINSON'S DISEASE

The tables 12 to 17 show the correlation between duration of Parkinson's disease, Heart rate variability parameters, Fasting insulin & HOMA-IR values. The correlation coefficients observed between the duration of Parkinson's disease & HRV parameters were negative and two of them were statistically significant. The correlation coefficients

observed between the HRV parameters and HOMA – IR values were all negative but none of them were statistically significant.

TABLE 12-CORRELATION BETWEEN DURATION OF PARKINSON'S DISEASE AND LF (ms^2)

	Correlation coefficient and p value	**Interpretation**
Correlation between duration of Parkinson's disease and LF (ms^2)	r=-0.7519 p=<0.0001***	Strong negative correlation and significant

*P value < 0.05 significant

TABLE 13-CORRELATION BETWEEN DURATION OF PARKINSON'S DISEASE AND HF (ms^2)

	Correlation coefficient and p value	**Interpretation**
Correlation between duration of Parkinson's disease and HF (ms^2)	r=-0.7532 p=<0.00001***	Strong negative correlation and significant

*P value < 0.05 significant

TABLE 14- CORRELATION BETWEEN DURATION OF PARKINSON'S DISEASE AND LF/HF RATIO

	Correlation coefficient and p value	**Interpretation**
Correlation between duration of Parkinson's disease and LF/HF ratio	r=-0.0156 p=0.937	weak negative correlation and not significant

P value < 0.05 significant

TABLE 15- CORRELATION BETWEEN HOMA – IR ,FASTING INSULIN AND LF (ms^2)

	Correlation coefficient and p value	Interpretation
Correlation between HOMA-IR and LF (ms^2)	r= -0.2718 p=0.14	Weak negative correlation and not significant
Correlation between Fasting insulin & LF (ms^2)	r= -0.2387 p = 0.20	Weak negative correlation and not significant

P value < 0.05 significant

TABLE 16- CORRELATION BETWEEN HOMA IR – FASTING INSULIN AND HF (ms^2)

	Correlation coefficient and p value	Interpretation
Correlation between HOMA-IR and HF (ms^2)	r= -0.1773 p=0.34	Weak negative correlation and not significant
Correlation between Fasting insulin & HF (ms^2)	r= -0.1792 p = 0.34	Weak negative correlation and not significant

P value < 0.05 significant

TABLE 17- CORRELATION BETWEEN HOMA IR ,FASTING INSULIN AND LF/HF RATIO

	Correlation coefficient and p value	Interpretation
Correlation between HOMA-IR and LF/HF ratio	r= -0.2078 p=0.27	Weak negative correlation and not significant
Correlation between Fasting insulin & LF/HF ratio	r= -0.1544 p = 0.41	Weak negative correlation and not significant

P value < 0.05 significant

5.4. SEVERITY OF PARKINSON'S DISEASE WITH HRV VARIABLES

The Heohn and Yahr scale is used in this study to assess the severity of disease. Based on Hahn and Yahr scale, patients are divided into three groups Group I (H&Y scale 1& 2), Group II (H&Y scale 3), Group III (H&Y scale 4,5). Analyses of variance for the effect of severity of disease on HRV parameters were worked out and the results were furnished in the tables 18 to 20. Highly significant difference was found among Group I (H&Y scale 1&2). Group II (H&Y scale 3) , Group III (H&Y scale 4, 5) with respect to LF (ms^2), HF (ms^2). The LF/HF ratio was not found to be different among three groups of severity of disease as per ANOVA.

TABLE 18 ANALYSIS OF VARIANCE FOR THE SEVERITY OF DISEASE ON LF (ms^2)

Study groups	n	mean±SD	Source of variation	df	Sum of squares	Mean Square	F value	P value
Group I	10	159.8± 51.9	Between Groups	2	57295.4	28647.7	18.546	0.000***
Group II	10	90.9± 39.43	Within Groups	27	41706.5	1544.69		
Group III	10	54.4±19.7	Total	29	99001.9			

*** Highly significant P < 0.001

Analysis of variance for the effect of severity of 3 disease groups based on H&Y scale on LF(ms^2) was significant with the p value 0.000

TABLE 19 ANALYSIS OF VARIANCE FOR THE SEVERITY OF DISEASE ON HF(ms^2)

Study groups	n	mean±SD	Source of variation	df	Sum of squares	Mean square	F value	P value
Group I	10	274.2±76.64	Between Groups	2	150600	75300	18.567	0.000***
Group II	10	161.3 ±69.97	Within Groups	27	109501	4055.5		
Group III	10	103.6±37.38	total	29	99001			

*** Highly significant P < 0.001

It is obvious that there was a significant difference among the three groups from the analyses of variance for the effect of severity of disease on HF (ms^2).

TABLE 20 ANALYSIS OF VARIANCE FOR THE SEVERITY OF DISEASE ON LF/HF RATIO

Study groups	N	mean±SD	Source of variation	df	Sum of squares	Mean square	F value	P value
Group I	10	0.5922±0.136	Between Groups	2	0.0084	0.0042	0.1709	0.84
Group II	10	0.598±0.16	Within Groups	27	0.6693	0.0247		
Group III	10	0.5598±0.174	total	29	0.6778			

P value < 0.05 significant

It is clear from the above table that there was no significant difference observed among the three groups for LF/HF ratio.

6. DISCUSSION

This study was aimed at evaluating the autonomic nervous system activity using Resting Heart Rate Variability and assessing the insulin resistance with serum insulin levels in Parkinson's disease patients. Resting Heart Rate Variability was performed to assess the integrity of sympathetic and parasymapathetic division of Autonomic Nervous System and to assess the sympathovagal balance, and thereby to assess the prevalence and pattern of autonomic dysfunction in the Parkinsonism patients. Insulin resistance measured using serum insulin levels might show its role in the etiopathogenesis of Parkinsonism disease by accentuating neurodegeneration in predisposed individuals.

6.1. CHARACTERISTICS OF STUDY SUBJECTS

The mean age of Parkinson's disease patients included in the study was 57 years who ranged from 48 years to 66 years. But, the mean age of Parkinson's disease patients in the earlier studies such as Dorji Hornod et al (2013)[156], T.szili Torok et al[107] (1999), T.H.Happaniemi et al (2001)[108] were 62.2 years, 66±7 years, 61.4 years respectively. Thus, the patients in the present study were in the age group lesser than the earlier studies.

Therefore, it could be stated that the changes in the Heart rate variability parameters are disease driven rather than age related.

The body mass index was calculated from the height and weight of the individual by the formula weight (kg)/ Height (m^2). None of the subjects in both the group were obese. The mean BMI of the Parkinson's disease group was 21.3 ± 1.71. The mean BMI of the control group was 22.08±1.68. The mean BMI of both the groups are not statistically significant so that it will not interfere with the dependent Heart rate variability variables according to Farah BQ Prado et al 2013 [163].

The duration of disease in Parkinson's disease patients included in this study was in the range between1 year to 16 years with the mean of 6 ± 4.64 years.

According to Charlotte A et al (2007) [157], the incidence of Parkinsonism in women is lower and age of onset is higher. This is possibly due to increased physiological striatal dopamine levels resulting from the activity of estrogen. In the present study, the Parkinson's disease patients were of almost equally distributed between sexes. Sexwise incidence of the disease was not seen.

6.2. RESTING HEART RATE & BLOOD PRESSURE IN PARKINSON'S DISEASE

The mean resting heart rate of the control was 78.03±5.91 and Parkinson's disease group was 74±7.6. The mean of the resting heart rate in parkinsons patients is little lower than the control group but, was not statistically significant. According to Soares et al (2013) resting heart rate was higher in Parkinsonism patients but did not differ significantly. Increase in resting heart rate might indicate the cardiovascular risk at earlier stage.

Both the systolic and diastolic blood pressure at rest was lower in Parkinson's disease group. The systolic blood pressure difference was highly significant (p value = 0.001) with mean systolic blood pressure of 113.06±12.11 compared to 120.86±4.50 of controls. Similar findings were observed in Brevetti et al (1990) [159], Barbeau et al (1969) [160], Szili-Torok T et al (1999)[107].

Brevetti et al (1990) [159] studied 24 hour blood pressure recording in Parkinson's disease patients. In their study, they recorded significantly low values of both systolic and diastolic blood pressure in Parkinsonism illness.

Barbeau et al (1969)[160] revealed that blood pressure in Parkinson's disease patients was usually lower than expected for age and sex which was resulting from diminished ability to secrete renin.

Gross et al (1972)[158] also demonstrated that in patients with Parkinson's disease mean blood pressure was lower than that of controls. However, M.J.Aminoff et al (1975)[161] reported contradictory findings stating that blood pressure was not low in Parkinson's disease patients.

Parkinson's disease is a progressive neurological disorder with pathological changes in the thalamus, corpus striatum, hypothalamic nuclei and brainstem reticular formation may lead to general lowering of both systolic and diastolic blood pressure.

In the present study, systolic blood pressure was significantly lower in Parkinson's disease patients than the control group suggesting the sympathetic hypofunction of heart in accordance with Barbeau et al (1969).

6.3. RESTING HEART RATE VARIABILITY

The present study was carried out to record the early changes in Autonomic nervous system induced by Parkinsonism using resting Heart

Rate Variability. Hence, the Parkinson's disease patients without any symptoms of autonomic dysfunction were enrolled for the study.

The Autonomic Nervous System is strongly influenced by sympathetic and vagus nerves. The resting Heart Rate Variability measurement signifies the autonomic tone. Under resting condition, cardiovascular system is under the control of both the division of Autonomic Nervous System. The extent of control by these two divisions varies from individual to individual. Spectral analysis applied to the inter beat interval has been considered as useful parameter for determining the Autonomic Nervous System functions. Heart rate variability is a valuable, non-invasive tool to assess the Autonomic Nervous System function. Any alteration in Heart Rate Variability is associated with the increased risk of adverse cardiovascular events.

Analysis of a Resting Heart rate Variability of 5 minutes recording done using the HRV analysis software, university of Kupio, version 1.1 among both the study groups showed the following results.

6.4. TIME DOMAIN MEASURES

Mean HR, Mean RR, SDNN, pNN50, RMSSD were the variable taken for the study as prescribed by the Task force.

6.4.1. Mean HR & Mean RR

The Mean HR was not significantly lower than the control group as shown in the Soares et al (2013). The average mean HR of the Parkinson's disease group was 72.33±6.8.Though these values are lower than the mean value obtained in normal subjects 76.8±6.04, but not statistically significant. The reduction in mean HR could be linked with the diminished baroreflex function as a result of vascular and cardiac sympathetic denervation in Parkinson's disease patients (Santiago Perez et al 2013) [109].

The mean RR is the mean of the selected RR series. The average value of the mean RR was 0.790±0.07 which was not significantly more than when compared to controls. This finding is in accordance with Dorji Harnod et al (2014)[156]. In contrast Szili-Torok T et al (1999) [107] recorded that the average RR interval shorter in Parkinson's disease patients.

6.4.2.SDNN

The SDNN is the standard deviation of Normal to Normal intervals which reflects the total power of spectral analysis. The mean value of the SDNN in Parkinson's disease group was 52.06 ± 26.16 which was significantly lower ($p = 0.03$) when compared to normal subjects. This finding is in agreement with the values of Soares et al (2013) [162]. The significantly reduced SDNN values signifies the reduced HRV in Parkinson's disease patients.

6.4.2. pNN50

In the present study, there was a reduction in pNN50 but not significantly. pNN50 was significantly less in Parkinson disease patients according to Soares et al(2013), M.Kallio et al (2000)[100], Szili-Tork T et al(1999) [107]. But, Van Dijik et al (1993)[116] found no significant difference in pNN50 between Parkinson's disease patients and controls.

6.4.3. RMSSD

This is most commonly used measure derived from the differences of NN interval. It is otherwise defined as the square root of the mean squared differences of the successive NN interval. RMSSD of short term

recording estimate High frequency variation in Heart rate and thus it is highly correlated with the HF.

The mean value of the RMSSD in our study in Parkinson's disease group was 34.05±14.77 which was significantly lower (p=0.03) when compared to normal subjects. The above observation is in agreement with the RMSSD values recorded by Szilli-Torok et al(1999) [107], Soares et al (2013) [162].However, M.Kallio et al (2000) [100] observed a reduction in RMSSD but do not differ significantly.

RMSSD, pNN50 are considered as sensitive indicator of parasympathetic function and thereby a low value indicates reduced vagal action. From the time domain measures, We observed a reduced Heart Rate Variability and reduced parasympathetic function in the present study.

6.5. FREQUENCY DOMAIN MEASURES OF HRV

Fast Fourier Transform method is followed in this study as it has the advantage of simple algorthims and a high processing speed. The power spectrum is commonly divided into ULF < 0.0033 Hz, VLF 0.0033- 0.046 Hz, LF 0.004-0.15 Hz, HF – 0.15-0.4 Hz (Task Force 96).

6.5.1. LOW FREQUENCY POWER LF(ms^2)

LF power component is primarily affected by sympathetic excitation and arterial pressure oscillation. The mean value of LF (ms^2) obtained in the Parkinson's disease patient was 101.7± 58.44 and that of control group was 171.033±97.6. The LF (ms^2) is significantly reduced in the Parkinson's disease group when compared to control group. This finding is in accordance with Dorji Hornard et al (2014)[156], T.H.Haapaniemi et al (2001)[112], Soares et al (2013)[162], Popisil P et al (2008)[104]. The above findings support the sympathetic hyopfunction in the Parkinson's disease patients. M.Kallio et al (2000), Van dijik et al (1993) observed a low values of LF (ms^2) but do not differ significantly.

6.5.2.HIGH FREQUENCY POWER HF(ms^2)

This power spectral oscillation indicates only the parasympathetic nervous system (i.e.) cardio vagal modulation and inspiratory inhibition of vagal tone. The mean value of the HF (ms^2) in the affected patients was 179 ± 94.7. These values are significantly lower than the values recorded in the normal subjects (266.13±170.74). The values are in agreement with the findings of Popisil P et al (2008)[104], Szili – Torok T et al (1999)[107],M.kallio et al (2000)[100], Soares et al (2013) [162]who reported significantly low nalues of HF(ms^2) in their studies. Dorji

Harnord et al (2014)[156] concluded that parasympathetic variable is more likely affected in Parkinson's disease patients. Van dijik et al (1993) [116] observed no difference between control and Parkinson's disease patients with respect to HF(ms^2). T.H.Happaniemi et al (2010)[108] recorded significantly low values of HF in th P value (0.004) in Parkinson's disease patients which is associated with the severity of disease.

6.5.3. LF/HF RATIO

LF/HF ratio, a noninvasive index of cardio sympathovagal balance is significantly decreased in this study. The LF/HF ratio obtained in Parkinson's disease patient was 0.584±0.53 and that of control was 0.719±0.26 with a p value of (0.01). Soares et al (2013)[162] observed a significant reduction in LF/HF ratio in Parkinson's disease patients reflecting the altered sympathovagal modulation in Parkinson's diseas patients. M.Kallio et al (2000)[100] found a significantly low values of AR derived LF/HF ratio which revealed the evidence of Autonomic Nervous System regulation failure. Popisil P (2008)[104] et al did not observe a significant difference in LF/HF ratio.

6.5.4. TOTAL POWER

Total power in the HRV is a sum of all the RR interval spectral powers (Very low frequency, Low frequency, High frequency) in the

short term recordings. It is nothing but the variance of all NN intervals. It estimates the overall changes in the heart rate due to cycles shorter than 5 minutes.

The mean value of the Total Power in Parkinson's disease group was 312.5 ±154.8 which was significantly lower than the controls (482.3± 267.5). Popisil P (2008) [104] et al found a statistically high significant decrease of Total Power (438 VS 1238 ms^2) in Parkinson's disease patients.

Therefore, the analysis of frequency domain variables revealed a reduced HRV suggesting autonomic modulation in the form of both sympathetic and parasympathetic dysfunction in patients with Parkinson's disease.

The neuropathology of Parkinson's disease involves both the sympathetic and parasympathetic nervous system because lewy bodies and neurodgeneration have been seen throughout the central autonomic centres in the hypothalamus, locus ceruleus,dorsal vagal nucleus, nucleus ambigus as well as in the intermediolateral coloumn cells in the spinalcord and in the postganglionic sympathetic neurons in addition to the dopaminergic pathway.

Thus, the cardiovascular dysregulation in Parkinson's disease could be related to above central or peripheral neurophysiology resulting in both sympathetic and parasympathetic dysfunction which is also supported by the results in the present study.

Reduced HRV and significantly reduced low values of time domain and spectral HRV measures observed in the present study suggesting the involvement of ANS in the Pathophysiological process of Parkinson's disease and these results are in accordance with the testing of other authors like Soares et al(2013)[162], Popisil et al (2008)[104], Dorji Harnord et al (2014)[156].

Sympathetic hypofunction in Parkinson's disease is supported by Turkka et al (1987)[114] who found out low values of serum nor epinephrine and its metabolite in Parkinson's disease patients. Impaired myocardial uptake of meta – (123I) iodobenzylguanidine (MIBG) in MIBG myocardial scintigraphy favours loss of cardiac sympathetic nerve terminal and gives evidence for sympathetic hypofunction in Parkinson's disease.(Goldstein DS, Holmes et al 1997)[101]

Presence of α-synuclein lewy bodies in dorsal vagal nucleus and higher centers cause parasympathetic nerve disturbances in Parkinson's disease patients. In parkinson's disease there is preponderance of

paraympathetically mediated disturbances has been shown by Piha et al 1988[164].

From the results, it is clear that both sympathetic and parasympathetic hypofunction is seen in Parkinson's disease patients. Significant reduction in time and frequency domain of HRV suggest that both sympathetic and parasympathetic influence on cardiac function are decreasing in Parkinson's disease patients. The time domain measures of HRV showing significant reduction in RMSSD, SDNN values and significantly low values of HF (ms^2) in the present study support reduced vagal action. It is obvious from the frequency domain measures showing significantly low values of LF (ms^2) LF/HF ratio revealed sympathetic hypofunction.

Reduced HRV and reduction in HRV indexes could be associated with the pathophysiology of Parkinson's disease and reflect loss of sympathetic and parasympathetic balance, which may be the result of neurodegeneration caused by Parkinson's disease itself.

There exists a close relation between autonomic dysregulation and the high mortality among the Parkinson's disease patients. Therefore, a prospective examination of autonomic regulation using HRV in Parkinson's disease might decrease cardiovascular risk and arrhythmia

related death in Parkinson's disease patients.(T.Szilli- Torok et al (1999)).

Cardiovascular autonomic dysfunction is relatively a under recognized problem of Parkinson's disease and it is closely associated with the progression of disease. Hence, the timely management of autonomic dysfunction might improve the patient's life quality and life span.

6.6. SERUM INSULIN LEVELS:

Serum insulin levels were measured using **Accubind ELISA microwells Insulin Test System** from the fasting blood samples. The mean value of the serum insulin levels in Parkinson's disease patients was 9.22±3.48 and that of control was 7.49±3.22 which was statistically significant (p value 0.04). The above values were in agreement with the findings of A.E.Boyd et al (1979)[16] and Domenico Bosco et al (2012)74, Sandyk et al (1993)[146].

The mean value of Fasting blood sugar in Parkinson's disease group was 103.5±11.3 which did not differ significantly when compared with the normal subjects. (p value 0.08)

6.7. INSULIN RESISTANCE (HOMA-IR)

HOMA-IR (Homeostatic Model Assessment of Insulin Resistance), a widely used method to measure insulin resistance was followed in this study. The mean value of HOMA – IR in the Parkinson's disease group was 2.30±0.70 which was significantly higher than the control group (1.71±0.61).

These results were in accordance with the findings of Domenico Bosco et al(2012)[74] , Morris JK et al (2013)[39]. Domenico Bosco et al (2012) stated that insulin resistance was present in 62% of Parkinson's disease patients of whom 36% had impaired glucose tolerance and 26% had only Insulin resistance. They concluded that there was significant positive correlation between Insulin resistance and Parkinson's disease with dementia.

Morris JK et al (2013)[39] found significant positive correlation between HOMA-IR values and dopamine depletion in both the substantia nigra and striatum in a preclinical model of Parkinson's disease. He reported that peripheral insulin resistance would accentuate the neurodegeneration in Parkinson's disease.

Multiple hits like oxidative stress, mitochondrial dysfunction, neuroinflammation play a role in the neurodegeneration of Parkinson's disease. Oxidative stress may be a connecting link between insulin resistance and neurodegeneration of Parkinson's disease.

According to M.Schaffer et al (1992)[134], Insulin acts as neuronal survival factor and protects against neuronal apoptosis. Moroo et al (1999)[147] reported that Insulin receptor mRNA declines within the substantia nigra which coincides with the loss of tyrosine hydroxylase , a rate limiting enzyme in the dopamine synthesis. Hence, the dopaminergic neurons are affected in the Insulin resistance.

Morris JK et al (2013)[39] suggested that Insulin resistance could make dopaminergic neurons in the substantia nigra more susceptible to toxic insults and exacerbate the process of neurodegeneration. Sandyk (1993) et al reported that impaired glucose tolerance out of insulin resistance may exacerbate the severity of illness and levodopa induced dyskinesia in Parkinson's disease.

Hence, the coexistence of insulin resistance in Parkinson's disease may increase their morbidity and progression of disease. Improvement of insulin sensivity and reduction of peripheral hyperinsulinemia through various measures like healthy diet, weight loss, aerobic exercise, drugs

may have beneficial effects in preventing or delaying the progression of Parkinson's disease.

6.8. CORRELATION OF VARIABLES

The negative significant correlation ($p < 0.0001$, $r = -0.7519$) was observed between LF (ms^2) and duration of Parkinson's disease. This finding is in accordance with the values of Dorji Harnod et al (2013) with a p value 0.04 and $r = -0.364$.

The negative significant correlation ($p < 0.00001$, $r = -0.7532$) was observed between HF(ms^2) and duration of disease. This finding is in agreement with the values of Dorji Harnod et al (2013) with a p value of 0.04 and $r=-0.356$.

Hence, both LF (ms^2) and HF (ms^2) showed significant negative correlation with the Parkinson's disease duration in this study. However, LF/HF ratio , a global indicator of sympathovagal balance showed a weak negative correlation with the Parkinson's disease duration in this study which was not statistically significant.

Therefore, the reduced HRV and low LF (ms^2) and HF (ms^2) values correlated with the duration of Parkinson's disease may be the

result of progressive neurodegeneration in the Parkinson's disease patients with the longer duration . (Dorji Harnod et al 2013)[156]

Both Fasting serum insulin and the insulin resistance index (i.e) HOMA-IR values showed a weak, negative correlation with LF (ms^2), HF (ms^2) and LF/HF ratio in this study. The reasons for not getting significant values could be attributed to small sample size covered in the study, variation among the diseased individuals.

However, the weak negative correlation coefficient for HRV variables and HOMA- IR supports the effect of insulin resistance on autonomic dysfunction. This finding is in agreement with Domenico Bosco et al (2012)[74] who found significant correlation between dementia and insulin resistance in Parkinson's disease patients. This finding is also strengthened by Liao DP et al (1991)[165] who stated that reduced HRV is associated with high insulin levels.

6.9. SEVERITY OF DISEASE WITH HRV VARIABLES

The patients classified into 3 groups according to the severity of disease showed significant variation in LF(ms^2), HF(ms^2) in this study with a p value < 0.000001.

There was no significant differences in the LF/ HF ratio values among the three groups I, II, III classified according to the severity of disease based on H & Y scale. Therefore, the severity of disease has profound influence upon the LF(ms^2), HF(ms^2) values in this study. However, the severity of disease showed no significant variation in LF/HF ratio though it showed much reduction in the severe group.

Obtaining the non significant value in LF/HF ratio could be attributed to small sample size, various confounding factors like drug, BMI & others.

This finding is in agreement with Pospisil P et al (2008) [104]who found a statistically decrease of Total Power, LF(ms^2), HF(ms^2) in the Parkinson's disease patients with a advanced PD (H&Y =2.3) than in mild PD(H&Y=1.3). For them also, the change of LF/HF ratio was not statistically significant.

All these findings suggest autonomic dysfunction from the reduced HRV indices and elevated Insulin resistance index values in this study may have a role in the etiopathogenesis of Parkinson's disease. A better understanding of the etiopathogenesis of Parkinson's disease might help in the management of Parkinson's disease and improvement of Patients

lifestyle and an early intervention may prevent the patients with Parkinson's disease from adverse cardiovascular events.

LIMITATIONS OF STUDY

The analysis of biochemical markers like Norepinephrine would have substantiated the autonomic dysfunction and their measurement could give valuable contribution to sympathetic hypofunction, which was not feasible in this study.

The confounding factors like drugs (eg . Levodopa, Selegilline) on Insulin resistance couldnot be avoided in this study.

Ultimately, an improved understanding of the role of autonomic dysfunction and insulin resistance in Parkinsonism patients may help to treat effectively the patients and improve their lifestyle.

7. CONCLUSION

Resting Heart rate analysis performed in Parkinson's disease patients have shown reduced HRV and both sympathetic and parasympathetic hypofunction in this study. This study concludes a reduced HRV from a significantly lowered values of SDNN, Total power and LF/HF ratio in Parkinson's disease group when compared to controls. In this study, sympathetic and parasympathetic impairement is supported by significantly decreased values of LF(ms^2), HF(ms^2) in Parkinson's disease group. Autonomic dysfunction in Parkinson's disease patients is significantly correlated with the Parkinsonian disease duration in this study. There is a significant difference found in the severe group according to H&Y scale with respect to LF(ms^2),HF(ms^2) in this study.

The insulin resistance index values are significantly high in Parkinson's disease patients when compared to controls. The prevalence of insulin resistance in Parkinson's disease patients is more when compared with controls in this study. Reduced HRV showed weak negative correlation with the insulin resistance in this study.

Thus, the HRV could be a potential tool to screen for autonomic dysfunction in Parkinson's disease patients and its timely management

might reduce adverse cardiac events in them. Insulin resistance could be a possible associated feature in Parkinson's disease patients which has effective role in the pathophysiology of disease.

Therefore, understanding the role of Autonomic nervous system and Insulin resistance in Parkinson's disease patients might help in the management of disease and prevent complications and improve prognosis.

8. SUMMARY

This study was performed to evaluate the autonomic function through resting Heart rate variability and to assess the role of insulin resistance in the pathogenesis of Parkinson's disease using serum insulin levels in comparison with normal controls.

30 patients with Parkinson's disease diagnosed as per Parkinson's disease brain bank criteria and 30 normal subjects as controls were subjected to Resting Heart rate variability. The serum insulin levels were measured by ELISA method. Both time and frequency domain HRV parameters showed significant variation when compared with the normal subjects. It is clearly found from the analysis of data in this study that Parkinson's disease patients had autonomic dysfunction with reduced HRV and both sympathetic and parasympathetic impairment, compared to their controls. The insulin resistance index values & fasting serum insulin levels were significantly higher in Parkinson's disease patients.

Thus, the Parkinson's disease patients are more prone for cardiovascular risk. Hence, the understanding of the role of autonomic dysfunction & associated Insulin resistance may help to prevent cardiovascular risk and the other complications of Parkinson's disease.

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INSTITUTIONAL ETHICS COMMITTEE
MADRAS MEDICAL COLLEGE, CHENNAI-3

EC Reg No.ECR/270/Inst./TN/2013
Telephone No : 044 25305301
Fax: 044 25363970

CERTIFICATE OF APPROVAL

To

Dr.S.P.Girijasivam,
Postgraduate
Institute of Physiology and Experimental Medicine,
Madras Medical College, Chennai-3.

Dear **Dr. S.P.Girijasivam,**

The Institutional Ethics Committee of Madras Medical College, reviewed and discussed your application for approval of the proposal entitled **"A Study of Heart Rate Variability and serum Insulin levels in patients with Parkinson's disease"** No.14042014

The following members of Ethics Committee were present in the meeting held on 11.03.2014 conducted at Madras Medical College, Chennai-3.

1. Dr. C.Rajendran, M.D, -- Chairperson
2. Prof. Kalaiselvi, M.D, -- Member Secretary
   Vice Principal, MMC, Ch-3
3. Prof. Nandhini, M.D, -- Member
   Inst. of Pharmacology, MMC, Ch-3
4. Prof.Bhavani Sankar, M.S, -- Member
   Prof & HOD General Surgery, MMC, Ch-3
5. Prof.V.Padmavathi, M.D, -- Member
   I/c. Director of Pathology, MMC, Ch-3
6. Thiru. S. Govindasamy, BA., BL -- Lawyer
7. Tmt.Arnold Saulina, MA MSW -- Social Scientist
8. Thiru.S.Ramesh Kumar, -- Lay Person
   Administrative Officer, MMC, Ch-3.

We approve the proposal to be conducted in its presented form.

Sd/Chairman & Other Members

The Institutional Ethics Committee expects to be informed about the progress of the study, and SAE occurring in the course of the study, any changes in the protocol and patients information / informed consent and asks to be provided a copy of the final report.

16/4/14.

Member Secretary, Ethics Committee

MEMBER SECRETARY
INSTITUTIONAL ETHICS COMMITTEE
MADRAS MEDICAL COLLEGE
CHENNAI-600 003

INFORMED CONSENT FORM

Title of the study: "A STUDY OF HEART RATE VARIABILITY AND SERUM INSULIN LEVEL IN PATIENTS WITH PARKINSON'S DISEASE"

Name of the Participant:

Name of the Principal Investigator: Dr.S.P.Girijasivam.

Name of the Institution:

Institute of Physiology and Experimental Medicine,

Madras Medical College and Govt. General Hospital,

Chennai - 3

Documentation of the informed consent

I _____ have read the information in this form (or it has been read to me). I was free to ask any questions and they have been answered. I am over 18 years of age and, exercising my free power of choice, hereby give my consent to be included as a participant in

"A Study of Heart Rate Variability and serum Insulin level in patients with Parkinson's disease"

1. I have read and understood this consent form and the information provided to me.
2. I have had the consent document explained to me.
3. I have been explained about the nature of the study.
4. I have been explained about my rights and responsibilities by the investigator.

5. I have been informed the investigator of all the treatments I am taking or have taken in the past _____ months including any native (alternative) treatment.

6. I have been advised about the risks associated with my participation in this study.

7. I agree to cooperate with the investigator and I will inform him/her immediately if I suffer unusual symptoms.

8. I have not participated in any research study within the past _____month(s).

9. I am aware of the fact that I can opt out of the study at any time without having to give any reason and this will not affect my future treatment in this hospital.

10. I am also aware that the investigator may terminate my participation in the study at any time, for any reason, without my consent.

12. I hereby give permission to the investigators to release the information obtained from me as result of participation in this study to the sponsors, regulatory authorities, Govt. agencies, and IEC. I understand that they are publicly presented.

13. I have understand that my identity will be kept confidential if my data are publicly presented.

14. I have had my questions answered to my satisfaction.

15. I have decided to be in the research study.

I am aware that if I have any question during this study, I should contact the investigator. By signing this consent form I attest that the information given in this document has been clearly explained to me and understood by me, I will be given a copy of this consent document.

For adult participants:

Name and signature / thumb impression of the participant (or legal representative if participant incompetent)

Name _____ Signature_____

Date_____

Name and Signature of impartial witness (required for illiterate patients):

Name _____ Signature_____

Date_____

Address and contact number of the impartial witness:

Name and Signature of the investigator or his representative obtaining consent:

Name _____ Signature_____

Date_____

INFORMATION TO PARTICIPANTS

Investigator: Dr. S.P.Girijasivam.

Name of Participant:

Title:

"A Study of Heart Rate Variability and serum Insulin level in patients with Parkinson's disease"

You are invited to take part in this research/ study /procedures. The information in this document is meant to help you decide whether or not to take part. Please feel free to ask if you have any queries or concerns.

You are being asked to participate in this study being conducted in

Institute of Physiology and Experimental Medicine,

Madras Medical College and Govt. General Hospital,

Chennai - 3

What is the Purpose of the Research?

Parkinson's disease is a neurodegenerative morbidity that leads to motor, psychiatric and sleep disorder characterized by motor symptoms, postural instability, shaking, rigidity, and slowness of movements . In addition to an extra pyramidal motor dysfunction, patients frequently show Autonomic Nervous System (ANS) disorders, even in early phases of the disease. Insulin resistance might potentiate the neurodegeneration in Parkinson's disease. Insulin resistance measured using serum insulin levels. High insulin levels ,insulin resistance are directly associated with autonomic dysfunction.

We want to assess autonomic functions in Paarkinson's disease patients and correlate the Insulin levels with autonomic functions in Pakinson's disease thereby to find a new approach in the treatment of Parkinson's disease.

The Study Design

Thirty patients with Parkinson's disease will be selected for the study.

Study Procedures

The study involves assessment of Heart rate variability and serum insulin levels.

You will be required to visit the hospital once during the study. 5ml of blood will be collected simultaneously during the study. Blood collection involves prick with a needle and syringe.

In addition, if you notice any physical or mental changes, you must contact the persons listed at the end of the document.

You may have to come to the hospital (study site) for examination and investigations apart from your scheduled visits, if required.

Possible benefits to you- autonomic dysfunction can be diagnosed at an early stage so that proper intervention can be taken.

Possible benefits to other people

The result of the research may provide benefits to the society in terms of advancement of medical knowledge and/or therapeutic benefits to future patients.

Confidentiality of the information obtained from you

You have the right to confidentiality regarding the privacy of your medical information (personal details, results of physical examinations, investigations, and your medical history). By signing this document, you will be allowing the research team investigators, other study personnel, sponsors, IEC and any person or agency required by law like the Drug Controller General of India to view your data, if required.

The information from this study, if published in scientific journals or presented at scientific meetings, will not reveal your identity.

How will your decision to not participate in the study affect you?

Your decisions to not to participate in this research study will not affect your medical care or your relationship with investigator or the institution. Your doctor will still take care of you and you will not loose any benefits to which you are entitled.

Can you decide to stop participating in the study once you start?

The participation in this research is purely voluntary and you have the right to withdraw from this study at any time during course of the study without giving any reasons.

However, it is advisable that you talk to the research team prior to stopping the treatment

PROFORMA

1. Name :
2. Age:
3. Sex:
4. Address :
5. Occupation :
6. Duration of parkinsonism :

7. History of Comorbid illness & duration :
   - Diabetes
   - Hypertension
   - Ischemic Heart disease
   - Cerebro vascular disease

8. Symptoms of autonomic dysfunction:
   - Giddiness on standing/near syncope
   - Palpitation
   - Abnormal/absence sweating
   - Gastroperesis /GERD
   - Bladder disturbances (retention, hesitancy, urgency, inconitence)
   - Bowel disturbances (constipation , diarrhea)
   - Loss of libido

9. History of any drug intake & duration

EXAMINATION

General examination:

Height

Weight

BMI

Waist –hip ratio

Pulse rate:

Blood pressure:

	Rt upper limb	**Lt upper limb**
Lying		
Sitting		
Standing		

Systemic examination:

Cardiovascular system:

Respiratory system:

Gastrointestinal system:

Central nervous system:

INVESTIGATIONS:

Hb

Fasting Blood sugar,

Post prandial Blood sugar

Blood urea , Serum creatinine

Fasting serum Insulin

HRV VARIABLES

FREQUENCY DOMAIN :

LF power

HF power

LF/HF ratio

TOTAL POWER

TIME DOMAIN :

Mean HR	Mean RR
SDNN	RMSSD
NN50	pNN50

MASTER CHART PATIENT

SL NO	AGE	SEX	BMI	RESTING HR	SBP RESTING	DBP RESTING	MEAN HR	MEAN RR	SDNN	pNN50	RMSSD	LF ms2	HF ms2	LF/HF ratio	TOTAL POWER	FBS	FASTING INSULIN	HOMA-IR	DURATION	H&Y SCALE
1	66	M	18.42	62	110	70	58	1.012	96	42.6	56.6	139	326	0.426	520	106	10.2	2.66	2	1
2	64	M	21.64	72	100	70	64	0.942	26	5.6	23.4	106	158	0.67	282	113	4.2	1.71	6	3
3	63	M	20.06	80	118	74	80	0.746	48	20.6	35.5	115	196	0.586	331	94	9.6	2.22	4	1
4	62	F	22.86	74	110	70	73	0.839	34	6.6	40.6	62	183	0.338	338	96	14.7	3.48	7	3
5	67	M	20.25	84	120	70	92	0.652	28	1.2	22.5	38	142	0.267	217	102	12.3	3.09	16	4
6	59	F	22.37	76	126	80	65	0.961	24	6.2	24.6	126	174	0.724	322	89	8.2	1.8	1	1
7	54	M	18.31	68	106	70	80	0.746	102	53.6	60.1	31	51	0.607	85	99	9.1	2.22	15	4
8	48	M	22.39	64	122	70	63	0.951	63	21	22.4	56	60	0.933	131	118	4.9	1.42	12	3
9	60	F	20.2	80	100	70	70	0.862	68	2.6	16.2	109	168	0.648	391	96	7.3	1.73	1	2
10	43	F	19.74	78	130	90	72	0.848	26	1.8	15.8	46	72	0.638	138	118	6.4	1.86	14	4
11	58	M	21.34	83	120	80	65	0.961	62	24.8	24.7	58	108	0.537	178	97	10.2	2.44	9	3
12	61	M	19.61	82	90	60	88	0.688	64	7.9	23.6	102	233	0.437	372	86	18.3	3.88	2	2
13	57	F	21.34	72	130	80	62	0.962	54	43.2	58.2	168	298	0.563	492	93	10.2	2.34	2	1
14	54	F	21.88	74	116	78	76	0.819	101	34.6	40.2	48	103	0.466	163	89	12.3	2.7	13	4
15	59	M	24.56	80	130	80	60	0.981	62	21.4	42.1	238	279	0.853	538	99	3.2	0.78	2	1
16	53	M	22.22	82	120	64	74	0.868	26	1.4	16.8	166	362	0.458	572	106	12.2	3.19	2	2
17	65	M	23.05	90	90	70	84	0.71	58	30.6	30.6	74	126	0.587	209	119	7.8	2.29	5	4
18	52	F	20.83	64	100	70	59	1.001	68	8.2	61.5	43	159	0.27	216	88	16.4	3.56	7	4
19	58	F	21.33	66	120	70	65	0.961	26	8.4	26.6	116	190	0.61	363	94	10.2	2.36	3	3
20	55	M	23.14	70	100	70	60	0.981	32	2.8	51.9	86	158	0.544	268	116	9.9	2.83	2	3
21	53	M	20.9	72	110	70	73	0.839	44	1.5	32.7	61	77	0.792	178	113	6.9	1.92	11	4
22	55	F	23.88	84	106	70	72	0.848	28	8.7	40.2	248	366	0.677	632	120	6.9	2.04	1	2

SL NO	AGE	SEX	BMI	RESTING HR	SBP RESTING	DBP RESTING	MEAN HR	MEAN RR	SDNN	pNN50	RMSSD	LF ms2	HF ms2	LF/HF ratio	TOTAL POWER	FBS	FASTING INSULIN	HOMA-IR	DURATION	H&Y SCALE
23	50	M	24.38	70	108	70	86	0.698	64	56.4	52.1	82	127	0.645	233	118	6.6	1.92	8	4
24	57	M	20.7	64	90	70	68	0.882	56	30.4	23.1	103	196	0.525	334	107	10.1	2.66	4	3
25	60	M	20.31	76	120	80	90	0.678	38	10.2	30.6	43	58	0.741	113	97	7.3	1.748	9	3
26	52	M	23.46	70	120	70	78	0.788	25	1	16.3	177	280	0.632	485	99	11	2.68	3	3
27	54	M	18.26	80	120	80	64	0.941	122	60.2	19.6	85	122	0.696	275	103	4.1	1.042	5	4
28	58	M	19.72	81	120	80	76	0.819	40	9.6	21.3	102	222	0.459	355	93	10.3	2.36	2	3
29	51	M	21.79	75	110	70	90	0.678	44	9.2	55.6	36	57	0.631	102	121	6.2	1.85	10	4
30	62	F	20.07	74	130	90	63	0.942	33	11.4	36.2	187	340	0.55	542	116	9.9	2.83	2	2

BMI	Body Mass Index
HR	Heart Rate
SBP	Systolic Blood Pressure
DBP	Diastolic Blood Pressure
SDNN	Standard Deviation of NN Intervals
LF	Low Frequency Power
HF	High Frequency Power
FBS	Fasting Blood Sugar
RMSSD	Root Mean Square of the Standard Deviation of NN Intervals
pNN50	Percentage of NN Interval differing by 50 milli seconds

MASTER CHART CONTROLS

SL NO	AGE	SEX	BMI	RESTING HR	RESTING SBP	RESTING DBP	MEAN HR	MEAN RR	SDNN	pNN50	RMSSD	LF (ms2)	HF (ms2)	LF/HF ratio	TP	FBS	FASTING INSULIN	HOMA-IR
1	52	M	22.66	82	118	74	63	0.951	103	38.2	79.3	128	187	0.684	370	97	6.3	1.5
2	54	M	20.57	76	126	70	70	0.824	42	14.4	16.8	240	229	1.049	587	68	7.9	1.32
3	55	M	21.68	82	124	80	76	0.802	32	11.6	29.1	133	451	0.295	642	97	5.4	1.29
4	53	F	20.46	81	124	84	72	0.806	67	22.8	52.7	132	120	1.095	289	107	7.8	2.06
5	50	F	24.68	79	116	76	68	0.91	58	20.6	77.1	85	112	0.761	212	113	5.4	1.5
6	55	M	21.05	74	114	68	80	0.704	96	31.8	30.8	277	348	0.795	669	80	12.2	2.4
7	59	M	23.12	78	124	76	70	0.823	114	38.6	16.8	43	59	0.729	111	73	11.1	2.01
8	57	F	22.43	81	110	70	80	0.746	54	17.2	17.4	323	416	0.776	801	86	9.4	1.99
9	56	M	25.97	96	124	76	72	0.806	41	14.6	37.9	188	308	0.610	550	118	5.4	1.57
10	58	F	21.22	74	120	80	81	0.714	116	29.4	70.1	72	76	0.946	156	122	4.2	1.27
11	45	M	20.74	80	122	78	74	0.823	110	24.6	26.3	71	95	0.747	187	92	11.3	2.56
12	48	M	23.11	60	124	84	70	0.862	64	21.2	36.2	145	153	0.946	330	83	10.2	2.09
13	42	F	22.31	70	124	80	76	0.787	142	46.8	75.5	423	551	0.770	1042	121	3.9	1.16
14	60	F	23.15	78	120	76	82	0.712	26	10.8	50.3	188	680	0.272	924	81	12.4	2.48
15	62	M	24.65	76	118	60	81	0.714	63	21.6	34.2	366	410	0.894	931	82	13.2	2.67
16	44	M	23.44	82	120	78	77	0.77	112	40.8	26.5	248	334	0.745	714	93	6.2	1.42
17	64	F	21.15	82	116	78	76	0.801	31	10.2	72.3	142	319	0.445	491	108	7.2	1.92
18	63	M	20.24	74	120	84	65	0.94	14	4.6	33.2	162	160	1.008	333	118	11.2	3.26
19	65	M	23.23	80	124	80	72	0.806	132	49.2	70.6	215	621	0.346	866	106	6.1	1.59
20	67	F	20.14	79	120	78	70	0.862	41	14.4	19.9	138	248	0.556	414	114	5.4	1.52
21	58	F	21.16	78	116	78	81	0.782	32	11.2	80.1	46	131	0.351	184	93	4.3	0.99

SL NO	AGE	SEX	BMI	RESTING HR	RESTING SBP	RESTING DBP	MEAN HR	MEAN RR	SDNN	pNN50	RMSSD	LF (ms2)	HF (ms2)	LF/HF ratio	TP	FBS	FASTING INSULIN	HOMA-IR
22	54	M	22.25	81	118	80	78	0.883	66	22.6	16.3	199	201	0.992	456	117	3.3	0.95
23	56	M	24.02	80	120	78	71	0.859	35	14.8	33.2	87	79	1.101	277	99	4.6	1.124
24	52	F	19.23	79	128	78	81	0.71	70.6	24.6	70.5	61	66	0.920	144	108	5.4	1.44
25	59	M	22.38	78	128	74	74	0.823	46	16.2	60.3	247	433	0.494	712	106	4.4	1.151
26	61	F	23.24	80	126	74	72	0.848	90	24.2	30.2	237	221	1.070	479	114	3.2	0.9
27	53	M	20.24	79	124	76	75	0.81	122	40.8	16.5	72	214	0.337	300	81	9.7	1.94
28	57	F	19.76	68	112	74	71	0.859	31	10.4	74.3	237	269	0.878	538	79	10.2	1.98
29	64	M	20.15	81	124	78	80	0.746	33	11.8	30.1	44	77	0.534	129	80	13.2	2.60
30	54	M	24.03	73	122	72	75	0.814	139	46.8	60.6	182	416	0.438	631	83	4.2	0.86

BMI	Body Mass Index
HR	Heart Rate
SBP	Systolic Blood Pressure
DBP	Diastolic Blood Pressure
SDNN	Standard Deviation of NN Intervals
LF	Low Frequency Power
HF	High Frequency Power
FBS	Fasting Blood Sugar
RMSSD	Root Mean Square of the Standard Deviation of NN Intervals
pNN50	Percentage of NN Interval differing by 50 milli seconds